Using Biological Tools to Assess Methadone Treatment

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Abstract

Methadone is acknowledged as an effective pharmacological substitution treatment for heroin dependence. However, patients presenting to addiction treatment services usually have multiple substance use, mental and physical health and social problems. Excessive alcohol use in individuals receiving methadone substitute maintenance treatment is a well-established clinical problem, which creates a treatment challenge. The research addressing the management of this group of patients is limited.

There were three key studies in this thesis. The first study investigated the measurement of methadone and EDDP in the urine samples of 60 patients, calculated the EDDP:methadone ratio, and explored whether it could be used effectively as an index of methadone metabolism among clients receiving methadone substitution treatment. The results indicated a correlation between methadone and EDDP concentrations and methadone dose; however, EDDP:methadone ratio exhibited a high inter- and intra-individual variability, which hindered the possibility of using it as a sensitive objective biomarker for monitoring compliance among patients receiving methadone. Part of the study examines whether this ratio is altered by the consumption of alcohol in a problematic manner by this group of patients. A small study also examined three patients during methadone induction, and examined EDDP and methadone ratio.

A further study explored the effectiveness of using the alcohol biomarkers ethyl glucoronide (EtG) and ethyl sulphate (EtS) to screen for recent alcohol consumption in 60 patients (138 urine samples) collecting their daily methadone dose. The results indicated that the EtG and EtS were sensitive biomarkers to detect alcohol use in the past 24 hours or more and therefore it would be a useful tool to incorporate during methadone treatment especially

coupled with knowledge of patients' co-dependence. The final study investigated the use of the breathalyser test in 23 patients who were screened for alcohol use before the prescription of methadone. Results found that breathalysers were successful in detecting alcohol use but for a much shorter timeframe than new recent alcohol biomarkers, which could offer a more specific and sensitive alternative.

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Abbreviations

ADH Alcohol dehydrogenase
ALD Alcoholic liver disease
ALDH Aldehyde dehydrogenase
ALT Alanine aminotransferase
AST Aspartate aminotransferase
AUDs Alcohol use disorders
BAC Blood alcohol concentration
CDT Carbohydrate-deficient transferring
EtG Ethyl glucuronide
EtS Ethyl sulphate
EtOH Ethanol
EDI Estimated daily intake of ethanol
FAEE Fatty acid ethyl esters
GC-MS Gas chromatography-mass spectrometry
GGT Gamma-glutamyl transpeptidase
HIAA Hydroxyindoleacetic
HTOL Hydroxytryptophol
LOD Limit of detection
LOQ Limit of quantification
LC-MS Liquid chromatography-mass spectrometry
MMT Methadone maintenance treatment
MCV Mean corpuscular volume
PEth Phosphatidyl ethanol
SCRAM Secure continuous remote alcohol monitor
SULT Sulfotransferase

Chapter 1 LITERATURE REVIEW

1.1 Methadone Biochemistry and Biological Markers

1.1.1 Introduction

The reduction of illicit drug use, the reduction of crime, and the improvement of health, including the control of the spread of blood-borne viruses, are important outcomes defining effective methadone treatment (Ward et al., 1999). Important factors during methadone maintenance have been addressed in the research and include methadone dose, treatment modality and level of service provision (Ball & Ross, 1991). Achieving and accurately measuring compliance remains a challenge for clinicians, as monitoring depends largely on self-report (Weiss et al., 2004; Wolff et al., 1999). However, the use of more objective measurements, such as biological markers, may provide an effective alternative or supplemental approach to assessing compliance. This research examined the potential usefulness of several such biological tools in connection with methadone maintenance treatment (MMT) in an outpatient facility.

1.1.2 Background

Illicit drug use affects users, their families, and society. It is a multi-dimensional problem that is estimated to cost the UK government over £15 billion in annual social, economic, health, and crime-related expenses (House of Commons Committee of Public Accounts, 2010). Currently, the government is estimated to spend £800 million annually on community-based drug treatment services, of which 30% is supplied by Primary Care Trusts (PCTs), which also provide over £200 million for alcohol treatment (National Treatment Agency for Substance Misuse, 2009; National Audit Office, 2008).

In the UK, almost one-third of the population admits to consuming illicit drugs at some stage in their lives; however, only a small percentage develops a so-called drug problem (NTA, 2009). For the period 2009-2010, there were an estimated 264,072 users of opiates and 42,078 users of crack cocaine in England, putting the former at roughly 7.7 per 1,000 of the population (Hay et al., 2013). The total number of users in both categories (306,150) had thus declined from the peak estimate of 332,090 in 2005-2006 – a claim that is supported by the decline in the number of heroin users seeking treatment for the first time from 2005-2006 (47,709) to 2011-2012 (9,249) (NTA, 2013). Nonetheless, the National Drug Treatment Monitoring System (NDTMS) reported in November 2013 that 193,575 drug users had been in contact with a drug service treatment in the previous year 2012-13. Of these, 80% were reported to be opiate users (NDTMS, 2013).

Heroin users are at risk of dying prematurely due to overdose. In fact, an overall pooled crude mortality rate among persons using heroin and other opioids in studies published between 1990 and 2008 was 2.09 deaths per 100 person-years, and was noted to be higher in males compared to females (Degenhardt et al., 2011). In 2012, 1,496 drug-related deaths were reported for England and Wales, of which 414 were linked to methadone (Office for National Statistics, 2013). The occurrence of any such deaths contributes to concern about the use of substitution therapy. However, it is understood that recognising risk factors can help prevent methadone related deaths. These risk factors include tolerance reduction, route of administration, gender, and history of non-fatal overdoses (Best et al., 2000; Bird & Robertson, 2011; Bird, Robertson, & Strang, 2010).

1.1.2.1 Methadone Treatment

Structured methadone maintenance treatment was first described in the United States by Dole and Nyswander (1965), who considered the pharmacological properties of methadone as an opioid receptor agonist to be highly useful in reducing illicit opiate (heroin) use (Dole & Nyswander, 1967). With the appropriate dosing to account for cross-tolerance with heroin, methadone would work by curbing withdrawal symptoms. This would assist clients to reduce their illicit drug use and engage with treatment (Kreek et al., 1973).

Initially, diamorphine was more heavily prescribed as a replacement opioid in the UK. However, the opening of a number of new drug treatment clinics in the late 1960s coincided with a shift to prescribing methadone to new clients, and by the 1970s most clinics were prescribing oral methadone as dispensed in the United States (Strang et al., 1994). Nonetheless, the number of opiate users increased steadily and was associated with a rise in crime over the next decade or so. As a result, in the 1980s the Advisory Council on the Misuse of Drugs (ACMD) recommended the expansion of community-based treatment and the development of Community Drug Treatment facilities (CDTs), the first of which was established in 1983 (Strang & Clement, 1994). Although there was a move toward short-term treatment owing to concerns about the spread of HIV among drug dependents using intravenous route, CDTs were encouraged to retain clients in long-term treatment (NTA, 2004). Indeed, the retention of clients in treatment was emphasized as a positive outcome for the better part of forty years, with general practitioners (GPs) responsible for a large share of methadone prescriptions (40% during the 1990s). However, in 2010 a new strategy was introduced that shifted the focus away from individual oriented care to the goal of achieving a drug-free life. The new recovery strategy emphasises sustained employment, improvement of mental health, and promoting good relationships with family and friends (Home Office, 2010).

Unlike Australia, Canada, France, the Netherlands, and Thailand, the approach to methadone treatment in the UK has depended on retail pharmacies to dispense prescribed doses of methadone (Gossop & Grant, 1991). The following section reviews dispensing practices in the UK, with a focus on issues surrounding supervised consumption.

1.1.2.2 Supervised methadone consumption in the UK

As noted above, obtaining a prescription to be dispensed in a retail pharmacy is a characteristic of the British methadone maintenance treatment system. However, concerns about methadone diversion and a reported increase of fatal methadone overdoses led to a global change in dispensing regulations in the 1990s (Cairns et al., 1996: Bell et al., 2010), and, in the UK, clinical practice turned to supervised methadone consumption in response to reports that the number of methadone-related deaths had begun to level with heroin deaths (Hickman et al., 2003; Strang & Clement, 1994). This trend began in Glasgow, but supervised methadone consumption began to appear in England in 1996 and, between 1999 and 2005, spread across the country (Lovell et al., 1999). During this period the Department of Health issued prescribing guidelines that recommended that clients should be dispensed methadone for consumption under supervision for the first three months in treatment ('Drug Misuse and Dependence: UK Guidelines on Clinical Management', 2007). In 2000 the ACMD recommended that supervised consumption be continued for the first six months of treatment (ACMD, 2000). In addition, supervised consumption is recommended whenever there is a break in treatment or when patients are considered difficult to contain or are perceived as 'chaotic' (Bell et al., 2010). Supervised consumption may be eased after a period of time or when a specified indicator of 'patient compliance' has been established – e.g., abstinence from illicit drug use (Robles et al., 2011). The community pharmacies remain the main dispensing body where supervised consumption takes place, and they engage in

more than 14 million face-to-face contacts with drug users every year (Matheson et al., 2007; NTA, 2006).

Strang et al. (2010) examined the impact of the introduction of supervised consumption on the rates of death due to methadone overdose in Scotland and England between 1993 and 2008. The authors found that deaths related to overdose of methadone peaked in the mid-1990s in both Scotland and England and subsequently declined. However, a slight increase was reported after 2000 in Scotland and in 2004 in England (Strang et al.).

1.1.3 Methadone pharmacology

1.1.3.1 Structure and chemistry

Methadone is a synthetic, long-acting pharmaceutical opioid that has been used as a substitution treatment in the management of heroin dependence since the 1960s (Beck et al., 2010). The U.S. Food and Drug Administration (FDA) approved methadone as a treatment for opiate dependence in 1972 (Kreek et al., 2010). The optimal dose of methadone can vary highly across patients, but it is nonetheless important to establish optimal dose to facilitate successful methadone maintenance treatment (MMT) (Lehotay et al., 2005).

Methadone is a weak basic drug with a molecular weight of 309.4. It has a pKa value of 9.2, making it a highly lipid soluble drug (Fredheim et al., 2008). Methadone's chemical structure of 6-dimethylamino-4,4-diphenyl-3-heptanone has a chiral centre that allows it to exist in two forms: *l*(R) and *d*(S) enantiomers (see Figure 1-1). Therapeutically, methadone is usually administered orally as a 50:50 racemic mixture. However, R-methadone is pharmacologically more active than S-methadone (Ferrari et al., 2004; Lehotay et al., 2005).

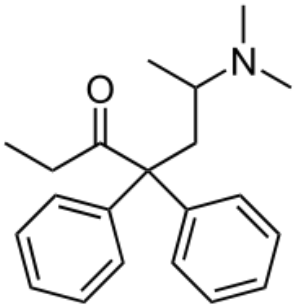


Figure 1-1 *Methadone: chemical structure with asterisk indicating the chiral carbon*

1.1.3.2 Stereoselectivity

The stereoselectivity of methadone influences its pharmacokinetic and pharmacodynamic properties. Essentially, the two isomers behave as separate substances and have their own kinetic profiles (Rentsch et al., 2002). However, these stereoselectivity differences have not been found to negatively impact therapeutic outcomes (Foster et al., 2000; Foster et al. 2004). In fact, studies have indicated the importance of stereoselectivity disposition in explaining the drug-drug interaction and concentration effects observed in studies of racemic methadone. For example, Eap et al. (1990) investigated protein binding and observed that R-methadone had lower plasma protein binding to alpha-1-acid glycoprotein than S-methadone, which could explain the various concentrations of plasma level and their importance when measuring methadone concentration levels (Eap et al., 1999). Eap and colleagues conducted a series of studies addressing the inter-individual variability of the plasma level of both R and S ratios of methadone. In 2000, Eap et al. looked at the plasma concentrations of the enantiomers of methadone and at the therapeutic responses of 180 MMT patients. The results indicated a large inter-individual variability in R-methadone concentration-to-dose-to-weight ratio, with doses of racemic methadone prescribed in a 70-kg patient varying from 55 mg/day to 921 mg/day. In an earlier study, Eap et al. (1996) looked at 22 patients receiving R-methadone maintenance treatment who were then switched to a double dose of R, S-methadone and observed a small amplitude decrease (16%). Finally, in a 1998 study,

methadone concentrations were found to vary over a sevenfold range in daily doses corrected for the body weight of patients receiving MMT (Eap et al., 1998).

Table 1-1 *Parameter Values (Mean) Describing the Kinetics of (R)-, (S)-, and (rac)-Methadone in MMT Patients and Healthy Participants (Population Analysis)**

Study	Number of participants (Treatment stage)	Vd (L)	t ½ (h)	CL/F (ml.min^{-1}. kg^{-1})
Foster et al., 2004	59 (Steady State)	112 (S)-methadone	39 (S)-methadone	2.02 (S)-methadone
		94 (R)-methadone	31 (R)-methadone	1.98 (R)-methadone
		145 (rac)-methadone	51 (rac)-methadone	1.98 (rac)-methadone
Boulton et al., 2001	8 (Single Dose)	227± 202 (S)-methadone	20 ± 4 (S)-methadone	4.95 (S)-methadone
		0.95± 78 (R)-methadone	43± 22 (R)-methadone	0.95 (R)-methadone
Wolff et al., 2000	17 (Single Dose)	220 (rac)-methadone	106.6 (rac)-methadone	0.99 (rac)-methadone
	19 (Steady State)	200 (rac)-methadone	32 (rac)-methadone	2.41 (rac)-methadone
	7 (Trough Plasma)	38 (rac)-methadone	45 (rac)-methadone	0.97 (rac)-methadone

*Vd= apparent volume of distribution; t ½ =elimination half life; CL/F=total oral clearance

Although previous studies indicated the importance of stereoselectivity in the pharmacokinetics of methadone, Foster et al. (1999) found that the metabolism of methadone via N-demethylation is not dependent on methadone's stereoselectivity. Instead, it is dependent on the CYP3A4 enzyme, and hence the inter-individual variability of methadone's pharmacokinetics could be related directly to the expression of CYP enzymes in individuals (Foster et al., 1999). However, in 2004 Foster et al. demonstrated that R-methadone has a greater elimination half-life and volume of distribution compared to S-methadone (Foster et al., 2004).

Wolff et al. (1997) observed an absorption lag time for methadone, S-methadone, and R-methadone in participants receiving higher doses, in addition to the difference due to stereoselectivity in the absorption process. The combined effects of this longer lag time and slower absorption rate meant that the time to reach peak concentration was 20 minutes longer for R-methadone than for S-methadone, while R-methadone's maximum concentration (Cmax) was on average 84% that of S-methadone. Furthermore, plasma binding studies found that S-methadone binds more readily to AAG protein than does R-methadone, which indicates that plasma protein binding is stereoselective. The methadone half-life is also influenced by its stereoselectivity (Wolff et al., 1997). In addition, Boulton et al. (2001) found that after a single oral methadone dose the half life of R and S methadone were 42.6 + h and 20.4 +- 4.0h, respectively. It is now established that S-methadone has a half-life of approximately 16 hours, compared to 36-48 hours for R-methadone (Ferrari et al., 2004). Researchers continue to debate the importance of using stereoselective methadone assays to determine methadone plasma concentration for methadone enantiomers in patients receiving MMT. However, in clinics, methadone is still administered orally in a racemic form instead of as R-methadone, which is more expensive to produce, despite the latter having been shown to be more active. Therefore, from a clinical practice standpoint, it is important to address the effects of methadone as a racemic mixture rather than in an R-methadone form. Nevertheless, it is worth noting that R-methadone, which is pharmacologically more active than the S-methadone compound, has been found to be a less significant factor in MMT outcomes than a CYP450 driven metabolism. Table 1-1 summarizes the pharmacokinetic parameters influenced by the methadone's stereoselectivity, including half-life.

1.1.4 Methadone pharmacokinetics

1.1.4.1 Absorption

Methadone is available in a variety of formulations, including tablets, injectable ampoules, and oral solutions (syrup or mixture). In the UK it is usually dispensed as an oral solution (1mg/ml) (NICE, 2007). Methadone's bioavailability varies from 41-76% to 85-95% (Eap et al., 1999) but has been described to be almost complete and relatively fast (Eap et al., 2002; Meresaar et al., 1981). Methadone is absorbed into the circulatory system and is detectable in plasma within 30 minutes, reaching peak plasma concentrations approximately three to four hours after acute administration, with concentrations levels maintained for 24 hours (Eap et al., 1999; Eap et al., 2002; Wolff et al., 1997).

Major determinants affecting oral bioavailability are intestinal first pass metabolism and extrusion of active methadone by P-glycoprotein (P-gp) (Kharasch et al., 2003). For example, Kharasch et al. (2004) investigated the role of hepatic and intestinal cytochrome CYP450, 3A and 2B6 in the metabolism, disposition, and meiotic effects of methadone and found that methadone had a low intestinal (22%) and hepatic (9%) bioavailability. However, it had a high oral bioavailability (70%), which also correlated with intestinal bioavailability. These findings confirm earlier studies (Oda et al., 2001; Kharasch et al., 2003) that indicated that first-pass intestinal metabolism was a determinant of methadone bioavailability (Kharasch et al. 2004).

1.1.4.2 Distribution

Methadone disposition is biphasic (i.e., following a bi-exponential curve). The transfer of the drug from the central compartment (blood) to the tissues corresponds to the fast alpha-phase. It is relatively constant because of the slow release from the hepatic store to the blood

(Novick et al., 1981). The high volume of distribution (Vd) has been documented as ranging between 0.16 to 6.7 (L/Kg) (Foster et al., 2004). Methadone is highly bound to plasma protein (over 90%) and, reportedly, up to 98% reaches the tissues due to high lipid solubility, leaving 1-2% of the free drug cycling in the blood. This facilitates the effectiveness of prescribing methadone once per day to MMT clients (Inturrisi et al., 1987; Kreek et al., 1973). Methadone is mainly bound to α_1-glycoprotein (AAG), although there is a five- to ten-fold variation in methadone binding in individuals (Wolff et al., 1991). Garrido et al. (2000) observed that patients with signs of withdrawal have shown higher levels of AAG, which could indicate that, in these instances, more of the methadone binds to proteins and less of the free drug is available to reach opioid receptors.

Plasma α_1-glycoprotein concentration has also been identified as a significant covariant for the apparent volume of distribution (Vd) of racemic methadone (Foster et al., 2004), which is consistent with its known role in determining the unbound fraction of the drug. Gender and α_1-glycoprotein concentrations explain over 50% of the variability in apparent Vd of methadone, with women reflecting a higher clearance of the drug compared to men (Foster et al., 2004). In addition, the binding of methadone is selective for the ORM2A variant (Eap et al., 1998) of AAG. Boulton (2001) found that plasma concentrations of the ORM2A variant of α_1-glycoprotein were a significant predictor of the inter-compartmental transfer rate for R-methadone. As such, methadone kinetics are characterized by substantial inter-individual variability in racemic methadone disposition.

1.1.4.3 Metabolism

CYP3A4 is involved in the N-demethylation of methadone to form 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP). EDDP is further N-demethylated to 2-ethylidene-1,5-dimethyl-3,3-diphenylpyraline (EMDP). Seven other metabolites have been identified;

however, due to the low concentrations that are produced, they are difficult to quantify and will not be discussed here. Inter-individual variability has been found related to CYP3A4 expression (Ferrari et al., 2004; Lugo et al 2005) and to other enzymes that play a less significant role than CYP3A4 in methadone metabolism, such as CYP 2B6, CYP 1A2, CYP 2C19, and CYP2D6. Foster et al. (2004) further reported that the polymorphic CYP3A5 isoform may also be important in methadone metabolism, as well as that CYP2D6 may be involved in the other oxidative pathways, especially for the metabolism of the R-methadone isomer (Kreek et al., 1979).

Although in vitro studies support the role of CYP2B6 in methadone metabolism, researchers have had difficulty supporting this finding through in vivo studies. Foster et al. (1999) indicated that the large variation in the pharmacokinetics may be due to the expression of these enzymes rather than to the stereoselectivity, as well as that CYP2B6 may metabolize S-methadone more rapidly than R-methadone. This finding led to the conclusion that the stereoselectivity of clearance is not due to n-demethylation partial intrinsic clearance. It remains unclear whether the pharmacokinetics or the enantiomer-specific metabolism is responsible for the clinical importance; together, however, they can help indicate the inter-individual variability in methadone pharmacokinetics (Totah et al., 2007).

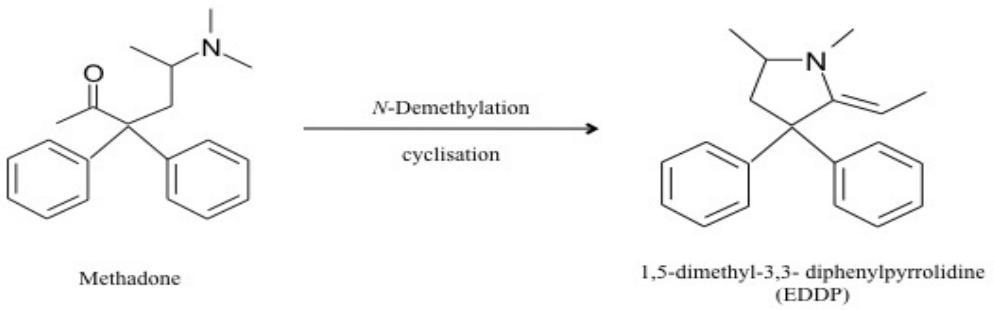


Figure 1-2 *Metabolism pathway of methadone*

1.1.4.4 Elimination and clearance

Methadone is eliminated by hepatic clearance followed by almost equal renal and faecal excretion. Forty-three percent of methadone is metabolised to the primary metabolite 1,5-dimethyl-3,3- diphenylpyrrolidine (EDDP), and a further 5-10% to 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP) (Bowen et al., 1978; Moffat et al., 1986; Sullivan & Due, 1973). The total combined urinary and faecal recovery of methadone and EDDP at steady state methadone maintenance accounts for 5-75% of the administered methadone dose, noting that elimination is mainly because of metabolic clearance (Nilsson et al., 1982). Although methadone metabolism is relatively normal in the presence of mild liver dysfunction, it decreases in clients presenting with severe liver disease (Novick et al., 1985). Alteration of the pH of urine plays a significant role in the elimination of unmodified methadone. Nilsson et al. (1982) found that both the half-life and volume of distribution of methadone increased two-fold when the pH of urine was increased. However, urinary pH is unlikely to have a clinical impact due to the small amount of methadone eliminated in urine (Anggard et al., 1975).

1.1.5 Methadone pharmacokinetic variability

The sequence of P-glycoprotein and the variability of the CYP enzyme activities probably influence the clearance and the plasma half-life inter-individual variability. Studies have shown the presence of CYP3A4 in both liver and intestinal mucosa, with a greater degree of variability in the former (Ketter, 1995). Studies have also indicated that CYP3A4 and, to a lesser degree, CYP2D6 are involved in methadone metabolism (Foster et al., 1999). Other enzymes, including CYP1A2 and CYP2C19, are also known to be involved in methadone metabolism (Shiran et al, 2009). However, further investigation is needed to determine the effects of genetically determined fast and slow metabolisers, which can have a direct effect

on the concentration of methadone in the plasma and thus could lead to inter-individual variation.

Studies have reported that there is a relationship between dosage and methadone plasma; however, the results are still conflicting. Some studies indicate that there is a linear relationship between the methadone dose and the plasma concentration (Wolff & Hay, 1994; Wolff et al., 2000). Moreover, although some studies have indicated that the relationship between plasma concentration and dose is explained by only 50 to 60% of the variability in concentration of methadone (Eap, et al., 2000; Foster, et al., 2000), other studies have focused on the inter-individual variability in plasma concentration (Kreek et al, 1973).

This variability of the methadone dose and plasma concentration is reflected in the difference in response within patients. Due to inter-individual variability, studies have shifted to investigating optimum methadone blood concentrations. Although 400 μg/L is considered the optimum blood level to stabilize maintenance, values vary between 50 and 600 μg/L. However, during therapeutic monitoring practice, 400 μg/L is the reference concentration used. For example, a study by Eap et al. (2000) investigated the blood concentration of R-methadone and its association to therapeutic response (defined as the lack of use of illicit drugs). The results reported large inter-individual variability in the therapeutic response for illicit opiate but not for cocaine. Although methadone is administered as a racemic structure, R-methadone is pharmacologically active and could be more important to measure in blood compared to S-methadone. In a recent study involving 180 patients receiving MMT, where the methadone dose ranged between 5 and 350 mg/day, therapeutic response was measured for both R- methadone (250 μg/L) and R, S-methadone (400 μg/L). The study indicated that R-methadone reflected a higher specificity threshold compared to R,S-methadone (Eap et al., 2000) The theoretical dose varied significantly, with between 55 mg/day and 921 mg/day

enabling the therapeutic response with levels of 400 ng/ml in a 70-kg patient receiving R,S-methadone (Eap et al., 2000).

Methadone elimination half-life can also be useful in evaluating the efficacy of methadone treatment. However, it is important to highlight that the methadone half-life is different for the first dose of methadone administered or as part of a steady-state dose/chronic methadone administration (see Table 1-1). This difference in the half-life is an indicator of a phenomenon known as auto-induction and was demonstrated by Wolff et al., (2000), who reported that the elimination half-life of methadone decreased from 128 hours at the start of treatment to 48 hours when methadone concentration achieved steady state; this was explained as due to the increase of the clearance to up to 350% due to CYP3A4 auto-induction. The change in the elimination half-life over time could be explained as the development of tolerance and could be considered an indication of the increase of the CYP3A4 metabolism. The increase of CYP3A4 activity has been reported to be highly variable: between five and twentyfold among patients (Boulton et al., 2001; Wolff et al.).

All of these factors indicate the influence of methadone dose and the importance of understanding that the variability in methadone pharmacokinetics indicates that some patients will require a higher or lower dose to avoid withdrawal symptoms. Indeed, it is clear that many factors influence the effectiveness of the daily dose of methadone and that a better understanding of these factors will help clinicians to achieve optimum dosage levels. This goal may be better achieved on an individual basis, taking into consideration the variability of methadone pharmacokinetics, which indicates that some patients will require a higher or lower dose to avoid withdrawal symptoms. In this regard, biological indicators, such as the methadone/EDDP ratio in urine, could serve as important tools in measuring individual metabolism of methadone and hence in assessing treatment compliance and effectiveness. As

the present study examined several potential biological indicators and sought to generate evidence regarding their potential applications, the remainder of this chapter is devoted to providing background on these potential tools.

1.1.6 Biological tools in methadone treatment

There are several biological indicators of methadone metabolism in the human body; however, quantities of these metabolites of methadone vary widely across the population and in response to the treatment received by patients. Moreover, although drugs can be identified in various bodily fluids, the identification and utilisation of biological markers of a given drug is highly influenced by its pharmacokinetics.

Blood has been the matrix of choice to give the most accurate measurement of the level of a drug in the body; however, urine provides a broad time frame for drug detection (Wolff et al., 1999) and hence has been the matrix of choice for drug testing to detect the use of illicit drugs. Furthermore, urine has been investigated as a matrix to verify patient compliance and to assist clinicians in adjusting methadone dosage by measuring its concentration alongside its inactive metabolite, EDDP (Goldstein et al., 2003).

In recent years, there have been efforts to optimise analysis methods for measuring methadone and its metabolites in urine, but the application of the relative concentration has been limited (El-Beqqali & Abdel-Rehim, 2007; Mandriolini et al., 2011). In urine, quantitative studies have documented concentrations of methadone and its main metabolite EDDP, and the relationship between the two compounds has been reported as a ratio. Although this ratio has been recommended as a potential tool for monitoring patient compliance, it is possible that the methadone/EDDP ratio could also indicate a metabolic rate.

As of yet, however, no reference range for this ratio has emerged for methadone in urine (Leimanis et al., 2012).

1.1.6.1 Indicators of methadone metabolism

Thus, urine has been a common matrix for drug testing in methadone treatment; however, its use as a means of monitoring compliance and dose adjustment has not been fully implemented. The main purpose of routine urine testing in patients receiving methadone treatment is to detect the use of heroin and other illicit drugs; however, other information can be determined by measuring the concentration of methadone and its main metabolite, EDDP, and establishing their ratio (Goldstein et al., 2003). In recent years, due to the limited clinical practice of methadone measurements in blood, there have been efforts to optimize analytical methods to measure methadone and its metabolites in urine (El-Beqqali & Abdel-Rehim, 2007; Holm & Linnet, 2012; Mandrioli et al., 2011).

1.1.6.2 Methadone and EDDP in urine

As noted, early studies documented urinary methadone and EDDP concentrations but failed to establish a reference ratio for EDDP/methadone. Purportedly, this is due to the high inter- and to some extent intra-individual variability of methadone and EDDP concentrations in urine (George & Braithwaite, 1999). In addition to investigating urinary methadone and EDDP, at least one early study also addressed the possible link between methadone concentrations in plasma and in urine. Using gas chromatography, Kreek (1973) investigated the methadone concentration in plasma and urine from nine patients receiving daily oral administration of 100 mg of methadone. The mean plasma methadone concentration was found to be 0.58 µg/ml, compared to 21.3 µg/ml in urine (Kreek et al.).

Kell et al. (1994, 1995) investigated the viability of estimating the methadone trough plasma

concentration from analysis of urine samples. Although the results were promising, the coefficient of variation for plasma concentrations at each prescribed dose was found to be large, which could be due to a poor dose response relationship in some cases or to other confounding conditions such as renal or hepatic diseases (Kell, 1994, 1995; Kell & Techman, 1996). Studies that examine methadone and EDDP levels in urine, however, have not been standardized compared to studies that have examined blood and plasma.The ratio between methadone and EDDP has been studied in various ways. For example, the Leimanis study (2012) aimed not only to investigate the metabolic ratio calculated as EDDP/methadone, but also to present a different relationship between the concentrations using a ratio calculated as methadone/EDDP**.**

Leimanis et al. (2012) investigated the relationship between methadone and EDDP in urine in order to develop a reference range as a tool for clinicians prescribing methadone for pain management. Ninety-five percent of the population had methadone concentrations between 0.175 and 20.9 mg/g cr, and on average EDDP twice the methadone concentration. Although the samples' methadone doses were not known, an attempt was made to control the variance due to different dose exposure by calculating methadone and EDDP from the narrow range of the excreted methadone. The study further investigated the relationship between EDDP and methadone by calculating the combined total of excreted methadone and EDDP; this enabled the calculation of methadone exposure, which is the combination of methadone and EDDP molar concentration. Methadone exposure in the inter-participant population ranged from 0.0832 to 229 mg/g creatinine (antilog –1.08 to 2.36), with median methadone exposure of 9.40 mg/g creatinine (antilog 0.973). Further investigation of the methadone and EDDP ratio was attempted by calculating the metabolic ratio (methadone/EDDP) and relating it to methadone exposure. The results indicated no relationship between the two parameters, with a 33-fold variability reported in the metabolic ratio (methadone/EDDP ratio) around the

median of the methadone exposure concentration (which was calculated in an attempt to predict the dose of methadone consumed). Therefore, the study concluded that due to the wide range of possible methadone exposure concentrations (which could mean that the urine samples analysed were collected from patients on a wide range of methadone doses), the metabolic ratio (calculated as methadone/EDDP) could not be reliably predicted (Leimanis et al.). Therefore, it is important to have a known methadone dose when analysing urine samples in order to reliably predict the methadone/EDDP ratio. Table 1-2 below documents the findings of the Leimanis et al. (2012) study.

Table 1-2 *Summary of Study Investigating the Levels of Methadone, EDDP and Other Indicators in Urine (Leimanis et al., 2012)**

Study	**Method of Analysis**	**Matrix**	**Time of Collection**	**Population**	**M Conc** Mean (mg/g cr)	**E/M Ratio** Mean	**E Conc** Mean (mg/g cr)
(Leimanis et al., 2012)	LC-MS	Urine	During routine clinic visits	Chronic pain patients Interparticipant population (8,083 participants and sample)	2.64	1.75	4.63
				Intraparticipant (190 participants) and (1,270 samples)	3.27	1.70	5.55

**M conc (methadone concentration), E/M ratio (EDDP/methadone ratio), E Conc (EDDP concentration).*

Studies focusing on developing analytical methods to measure methadone and EDDP have also documented the relationship between them. However, the purpose of these studies was not to establish the metabolic ratio but rather to establish a relationship that was expressed as a ratio by calculating methadone/EDDP. Angelo et al. (1999) conducted a study designed to develop a high performance liquid chromatography method to analyse methadone and its metabolite in urine. The study did not document the doses of methadone in the samples collected; however, the mean ratio of EDDP/methadone was 3.2, and the range varied between 1.3 and 12.7 in 21 urine samples collected from MMT patients (Angelo et al.).

Previously, Lanz & Thormann (1996) examined the EDDP/methadone ratio while investigating the capillary electrophoretic assay. The reported mean urinary ratio of EDDP/methadone was 0.63, ranging from 0.36 to 2.92 in eight MMT patients. However, no information on the doses or the time spent in treatment was referenced in the study. Still earlier, a number of researchers documented observations in relation to the ratio, such as Verebely et al. (1975), who investigated the EDDP/methadone ratio in 12 patients who were just beginning to receive methadone maintenance. The findings indicated that the EDDP/methadone ratio increased from 0.6 to 1.8 after 26 days of receiving methadone doses every day (Verebely et al.).

Most recently, Nielsen et al. (2013) investigated the potential of measuring the metabolite/drug ratio (EDDP/methadone ratio) in post-mortem blood or urine to indicate whether cause of death was due to treatment non-compliance. The study included taking measurements of EDDP/methadone in living persons receiving methadone. The median of the EDDP/methadone ratio was 1.6 (0.45-5.4). This ratio was not significantly different when compared to the ratio in post-mortem samples (Nielsen et al.).

1.1.6.3 Excretion of methadone and EDDP in urine

As a basic compound, the elimination of methadone is known to be affected by pH. Nilsson et al. (1982) found that both the half-life and the volume of distribution of methadone increased two-fold when the urine pH was increased. In terms of the EDDP/methadone ratio, when there was an acidic environment, the ratio decreased and the renal clearance of methadone was three times greater (Bellward et al., 1977).

More recently, Bernard et al. (2007) examined the relationship between methadone and EDDP and the effect of pH. Urine samples (n=1539) that had tested positive for methadone

were provided from the Division of Forensic Toxicology and Drug Abuse (DFTDA). However, no records on the dose or the length of time for which methadone was prescribed were collected. The results indicated that the median methadone level was 27.3 μmol/L (range: 0.7–258.7) and the median concentration of EDDP was 42.6 μmol/L (range: 0–244.8). The ratio between methadone and EDDP concentrations was calculated and plotted against pH to test whether it varied with pH. The results indicated a good relationship between the ratio and the pH (Spearman's $\rho = 0.7$, $P < 0.01$), confirming that methadone but not EDDP is pH-dependent. The study also indicated that some of the samples with positive methadone and negative EDDP may have been spiked, and some results may have been due to this adulteration rather than pharmacokinetic reasons and/or inter-individual variation (Bernard et al., 2007).

Larson & Richards (2009) investigated the potential for developing a regression model that could predict patients' drug adherence based on the ratio of EDDP/creatinine levels in urine. Urine was collected from a total of 40 patients receiving methadone, either for heroin dependence treatment or chronic pain. The doses of methadone consumed by the patients varied between 10mg per day and 125mg per day. The levels of creatinine, methadone, and EDDP were all measured in urine using GC/MS. Although the results indicated some variability in the EDDP concentration levels in urine, correction for creatinine was effective in removing the substantial variability. The results indicated that the ratio of EDDP/creatinine was found to predict the consumed dosage of methadone (Larson & Richards).

A number of studies have investigated the importance of understanding the effect of metabolism of methadone on the concentration of methadone and its metabolite in plasma (Goldberger et al., 1994; Seldén et al., 2012; Verebely et al., 1975). Byrne et al. (1998) investigated the use of weekly trough serum levels for optimising methadone dose and found

that increasing the dose when concentrations were less than 200 μg/L helped to reduce illicit drug use and increased treatment efficacy. These findings indicate the importance of methadone level as an indication of treatment efficacy (Byrne et al.).

Table 1-3 *Summary of Studies Investigating Methadone and EDDP in Urine**

Study	Method of Analysis	Time of Collection	Population	Dose (mg/day)	Methadone Conc	M/E Conc	EDDP Conc USE ug/L
George et al. (2000)	GC-MS	Random	38 specimens from routine drug of abuse screening	Not reported			52 to 515 ng/mL
George et al. (1999)	GC		14 participants during detoxification	30-100	11.8 ng/mL	Not reported	17.8 ng/mL
Preston et al. (2003)	GC-MS	MMT patients, 3 times a week at trough	27 participants at stabilization (first 5 weeks)	35-80 same here	Least-square mean= 5913ng/mL	Least-square mean= 1.49	Least-square mean= 4507ng/mL
			27 participants at steady state (12 weeks)	35-80	Least-square mean= 6691ng/mL	Least-square mean= 1.71	Least-square mean= 4754 ng/mL
Bezie et al. (2004)	Immuno-assay		68 patients and 1917 urine samples	Average dose 65 mg	600 to 300 AU		

**M Conc (Methadone concentration), E/M ratio (EDDP/Methadone ratio), E Conc (EDDP concentration*

A pilot study by George et al. (1999) investigated the application of urinalysis during detoxification to measure compliance using methadone to EDDP ratio in urine. The study investigated the relationship between the dose of methadone and the concentration of methadone and EDDP in urine in patients undergoing methadone detoxification. The results indicated that although there was a significant relationship between the dose and the concentrations of methadone and EDDP in urine, there was high inter-individual variability. Correcting for creatinine concentration did not improve the results; however, the authors suggested monitoring the excretion patterns of methadone and EDDP in each individual (George et al.) (see Table 1-3).

It is also important to consider the phase of methadone treatment at which the methadone concentration is measured. Nilsson et al. (1982) found that the pH of urine was different among patients receiving methadone at induction (1-3 days) and later (24-26 days). This change in pH influenced the rate of clearance by 20% and was found to increase from (0.095 ± 0.031L.min compared with 0.115 ± 0.036 L.min). The authors suggested that this phenomenon could be explained by acidosis, induced by methadone due to respiratory depression. Therefore, when choosing urine as the matrix for measuring the concentration of methadone, pH has a large impact on inter-individual variability and needs to be regulated or measured upon analysis of the sample.

Studies have documented the concentrations of methadone and EDDP in urine, and recent research has focused on the development of techniques to measure these concentrations in various biological matrices. The clinical use of these concentrations has also been suggested, including for monitoring compliance. Although some studies attempted to investigate the relationship between methadone and EDDP as a ratio,

only one study has investigated the development of a reference range for the (metabolic) methadone/EDDP ratio in pain patients (see Table 1-2), the results indicated a high variability in concentrations of EDDP and methadone and therefore in the ratio (methadone/EDDP) as well, which the authors attributed to the lack of methadone dose information. Therefore, the present research aimed to investigate the methadone/EDDP ratio in relation to known doses and times of sample collection.

1.2 Alcohol Biochemistry and Biomrkers

1.2.1 Introduction

The hazardous and harmful use of alcohol and dependence is a major global contributing factor in death, disease, and injury (Rehm et al., 2009). In 2007, the World Health Organisation reported that the harmful use of alcohol resulted in approximately 2.5 million deaths each year (WHO, 2007). By 2012, the estimate had risen to 3.3 million deaths (WHO, 2012). Moreover, as a risk factor for global burden of disease (GBD), alcohol is ranked fifth, behind tobacco (Ezzati et al., 2004). Nutt et al. (2010), using a multicriteria decision analysis (MCDA) approach to modelling, found alcohol to be the most harmful of all the drugs considered. According to data cited by Rehm et al., alcohol is implicated in 3.8% of all global deaths and is responsible for 4.6% of global disability-adjusted life years (DALYs). This strain on global wellness also places a burden on productivity and healthcare costs. In the UK alone, the cost of alcohol misuse is estimated at £6.4 billion annually, with £3 billion of this cost borne by the National Health Service (NHS) (Ward et al., 2010).

Faced with the dramatic impact of alcohol misuse on societies, politicians at the community and national levels, as well as health authorities worldwide, have pushed

for more effective screening and treatment of alcohol use disorders (AUDs). AUDs are complex biopsychosocial conditions (Li et al., 2007) that can manifest in a variety of ways. For descriptive purposes, however, it is useful to recognize at least the following broad categories or levels of abuse: *hazardous drinking*, *harmful use*, and *alcohol dependence*. Definitions of these conditions are provided in Table 1-4.

Table 1-4 *Types or Levels of Alcohol Use Disorder (adapted from WHO, 2001)*

Category	**Definition**
Hazardous drinking	A pattern of alcohol use that increases the risk of harmful consequences for the user. In contrast to harmful use, hazardous use refers to patterns of use that are of public health significance despite the absence of any current disorder in the individual user.
Harmful use	A pattern of alcohol use that is causing damage to health. The damage may be physical or mental. Harmful use commonly, but not invariably, has adverse social consequences.
Alcohol dependence	A cluster of behavioural, cognitive, and physiological phenomena that develop after repeated alcohol use and that typically include a strong desire to take alcohol, difficulties in controlling its use, persisting in its use despite harmful consequences, a higher priority given to alcohol use than to other activities and obligations, increased tolerance, and sometimes a physical withdrawal state.

A range of pharmacological and/or behavioural interventions exists for the treatment of AUDs (Li et al., 2007). However, the implementation of these treatments – as well as the study of these conditions and the identification of individuals misusing alcohol – requires reliable screening tools. To screen for current and/or recent alcohol use or misuse, most clinical and research settings make use of clinical histories, physical examinations and self-reported questionnaires such as the Alcohol Use Disorders Identification Test (AUDIT), the CAGE (Ewing, 1984) and the Michigan Alcoholism

Screening Test (MAST) (Selzer et al., 1971). However, the accuracy of self-reported measures such as these can be affected by under-reporting and/or under-estimation of harmful drinking (Del Boca & Darkes, 2003).

In contrast, bio-physiological markers of the presence or recent presence of alcohol in the human organism (i.e., alcohol biomarkers) can provide the basis for a variety of objective methods to detect and assess alcohol consumption (Johnson et al., 2008; Litten et al., 2005). Although some of these methods still require further research, they offer promising options for evaluating the efficacy of treatment and identifying individuals who are under the influence and/or at risk of alcohol misuse. Outside of clinical or research settings, potential applications for these modalities include the screening of motor vehicle operators and workplace drug testing (WDT).

The current standard for assessing recent alcohol consumption, which involves measuring alcohol in the breath, blood, or urine, can only detect consumption within the previous 12 hours. Methods involving alcohol biomarkers have the potential to be more reliable than standard methods and/or to be reliable over longer periods. Certain alcohol biomarkers have been used to measure consumption in excess of 1000g of ethanol over periods (detection windows) of more than two weeks (Helander et al., 2009; Hoiseth et al., 2008). This chapter focuses on the potential use of two biomarkers that appear to offer broad detection windows: ethyl glucuronide (EtG) and ethyl sulphate (EtS). Following a brief overview of alcohol and alcohol metabolism, the chapter addresses some of the research carried out on these and other biomarkers to date and discusses the available evidence regarding the potential of EtG and EtS for use in screening for alcohol consumption.

1.2.2 Problematic alcohol consumption and alcohol dependence

A better understanding of what distinguishes alcohol dependence from problematic alcohol consumption is important in order to effectively treat patients presenting to drug treatment facilities, as recognizing the problem early in the treatment can help clinicians to develop better intervention measures and thus to avoid escalation of the problem. Indeed, management of dependence presents a crucial period in the treatment of severely alcohol dependent patients due to the life threatening conditions caused by alcohol withdrawal symptoms that can develop when users suddenly discontinue or decrease their alcohol consumption (Leggio et al., 2008). However, in many cases alcohol problems receive little attention in the treatment of illicit drug misusers (Gossop et al., 2005).

The two most prominent classification systems, those of the Diagnostic and Statistical Manual (DSM-IV) and the International Classification of Diseases (ICD-10), agree in defining 'alcohol abuse' and 'harmful alcohol use' (their respective terms) as a repetitive administration of alcohol that results in harmful consequences to physical or psychological health. However, ICD-10 excludes the use of negative social consequences in defining 'harmful alcohol use' (Finch & Welch, 2006; Nelson et al., 1999). Both classifications view alcohol dependence similarly, and both apply three criteria that must have been met within the previous 12 months in order for an individual to be diagnosed with alcohol dependence. In developed countries, the application of these classifications indicates that almost 5% of the population suffers from alcohol dependence, compared between five and 15% presenting with hazardous or harmful drinking (Saunders & Lee, 2000).

1.2.3 Alcohol and alcohol metabolism

Alcohol, or technically ethanol (EtOH), is a psychoactive substance, the consumption of which can lead to dependence (Rehm et al., 2007). It is a polar molecule with a weak charge and a low molecular weight. It is absorbed rapidly on ingestion, with absorption completed in one to three hours, with ~20% absorbed from the stomach and the rest from the small intestine (Jones, 1996).

Alcohol is typically consumed in beverages such as beer, wine, and spirits. Alcohol content in such products is usually expressed in terms of percentage of alcohol by volume (ABV). For example, beer identified as 9% ABV contains 9 units of ethanol for every 1000 mL of beer (1 unit of alcohol is equivalent to 8g of pure ethanol). In the 1980's, the medical Royal Colleges endorsed the Health Education Council's guidelines, which recommended that men should consume no more than 21 units (168 g) and women no more than 14 units (112 g) per week (House of Commons Science and Technology Committee, 2011). Table 1-5 shows the descriptive terminology associated with various levels of alcohol consumption, correlated with ranges expressed in terms of such units. These ranges can serve as a reminder that, in assessing alcohol consumption in relation to health and social consequences, it is usually important to take account of such factors as gender, body weight, and alcohol tolerance, as well as the time over which a given amount of alcohol is consumed, as these factors can play a role in the physiological and/or psychological impact of alcohol (Rehm et al., 2009).

Table 1-5 *Descriptive Terms for Alcohol Consumption Levels Correlated with Ranges Expressed in Standard UK Units* (Agarwal, 2002; UK Department of Health, 1992)

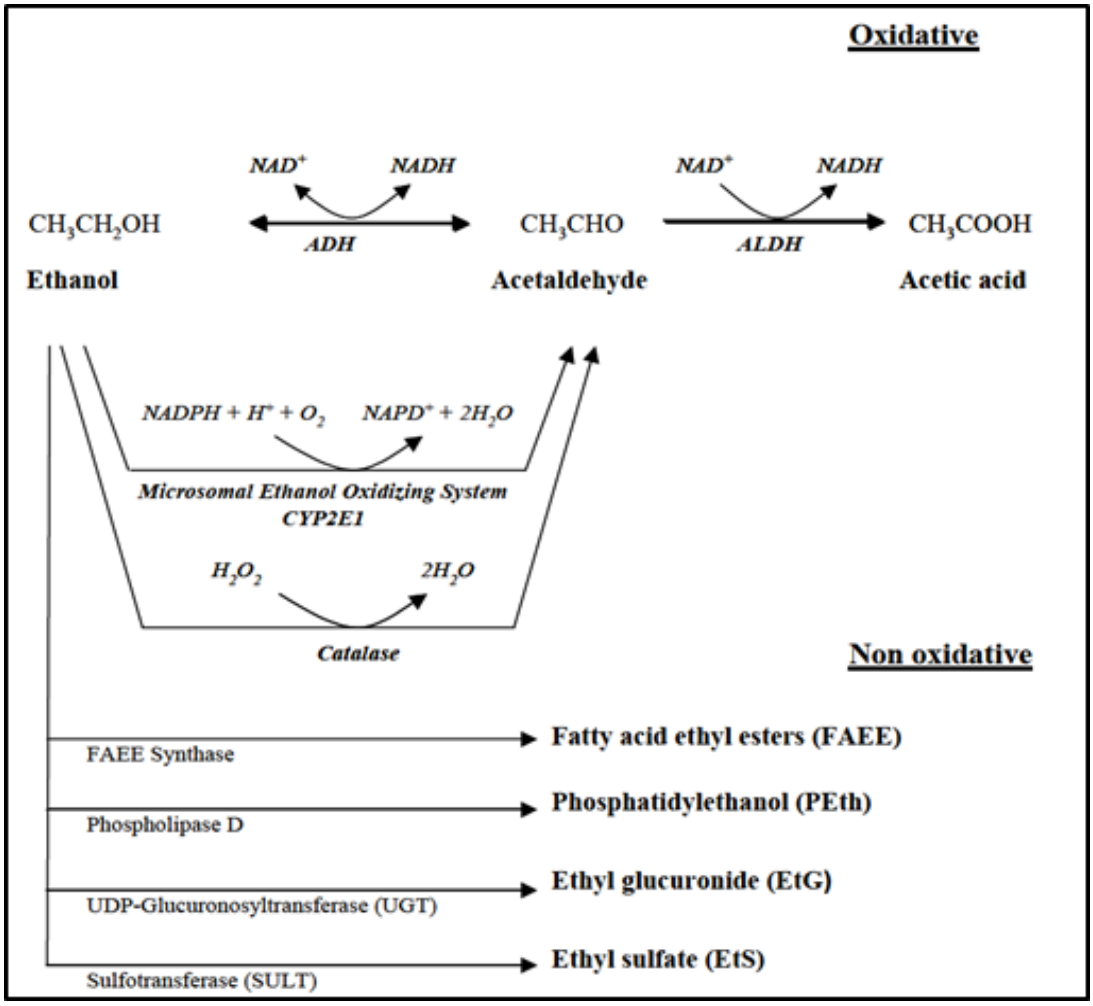
Descriptive Term	**UK Units/day (1 unit = 8g of pure ethanol)**
Heavy	>10
Sessional	>6-8
Moderate	4-10
Light	<4

Alcohol is metabolised primarily in the liver, in a two-stage oxidation process: first to acetaldehyde by alcohol dehydrogenase (ADH) and then further to acetic acid by aldehyde dehydrogenase (ADH). The majority of ingested ethanol (~95%) is metabolised by the liver via alcohol dehydrogenase (ADH) into an acetaldehyde. A smaller amount is oxidized into aldehyde by catalase or by the enzyme CYP450 in the microsomal oxidising system (MEOS), as shown in Figure 1-3. It is further oxidized to acetic acid by ALDH.

1.2.3.1 Oxidative pathway

The main oxidative metabolism of ethanol takes place in the cytoplasm of the hepatocyte by ADH, through the action of nicotinamide adenine dinucleotide (NAD), and the acetaldehyde is further oxidized to acetate by NADH (Sato & Kitamura, 1996). The isozyme pattern and activities of ADH and ALDH have been described in the human mouth, oesophagus, stomach and large intestine (Dong et al., 1996; Yin et al., 1993, 1994, 1997).

Figure 1-3 *Ethanol elimination pathways (Dahl et al., 2006)*



The cytochrome P450 iso-enzymes, which include CYP2E1, 1A2, and 3A4, are also part of the oxidative metabolism process in the liver; however, CYP2E1 plays a much more substantive role in the MEOS than CYP1A2 or CYP3A4 (Asai et al., 1996; Niemela et al., 1999; Salmela et al., 1998). Chronic alcohol consumption has been found to induce the main enzyme, CYP2E1, to metabolise ethanol to acetaldehyde at high ethanol concentrations, which thus leads to an increase in the MEOS activity (Lieber, 1989; Lieber & DeCarli, 1968). Although CYP2E1 is the main enzyme, it has been proposed that chronic alcohol consumption leads to the induction of CYP1A2

and CYP3A4 and may contribute to the pathogenesis of liver damage due to the generation of reactive oxygen species (ROS), which is key for the progression of fatty liver to steatohepatitis.

One of the reasons for the increase in CYP2E1 protein during chronic ethanol intake is decreased proteasomal degradation, which increases CYP2E1 protein stability. The induction of CYP2E1 occurs within one week of the consumption of ethanol, even at doses as low as 40g/day in chronic alcohol consumers. Jones et al. (1992) identified the rate of ethanol elimination as 0.21/g/L/h. However, the degree of induction varies highly across individuals (Jones et al., 1992). Catalase is another enzyme that, in the presence of a hydrogen peroxide (H_2O_2)-generating system, is capable of oxidising ethanol. This, however, is considered a minor pathway of alcohol oxidation, except in a fasting state (Handler & Thurman, 1990).

Regardless of the path by which ethanol is oxidised, the end products are acetaldehyde and acetate. Acetaldhyde is highly toxic and is metabolized rapidly – mainly by aldehyde dehydrogenase (ALDH) to form acetate, which requires the coenzyme nicotinamide adenine dinucleotide (NAD^+), which is later reduced to NADH. Acetate is oxidised away from the liver, yielding the end products CO_2 and H_2O in the cells of the heart, skeletal muscles, and brain (Krebs' cycle). Acetate is also metabolised into acetyl coenzyme-A, which is involved in the lipid and cholesterol biosynthesis of the brain peripheral mitochondria. In chronic alcohol consumers, it has been suggested that acetate is used by the brain as a source of energy instead of glucose (Zakhari et al., 2006).

1.2.3.2 Non-oxidative metabolism

Between three and ten percent of ingested ethanol is excreted via the breath, sweat, and urine (Jones, 1990; Jones et al., 2011). A much smaller percentage (<0.1%), however, undergoes non-oxidative metabolism, or phase II conjugation (Helander et al., 2009; Helander & Beck, 2004; Hoiseth et al. 2008). The conjugation of ethanol with glucuronic acid is catalysed by endoplasmic reticulum uridine diphoshate – glucuronyltransferase (UGT), which produces ethyl glucuronide (EtG). Ethyl sulphate (EtS), on the other hand, is produced by the conjugation of ethanol with sulphate, which is catalysed by cytosolic sulphotransferase (SULT). Other end products of this metabolic pathway include Fatty acid ethyl esterase (FAEE) via FAEE synthase and Phosphatidylethanol (PEth) via phospholipase D (see Figure 1-3).

1.2.4 Biological tools

1.2.4.1 Alcohol Biomarkers

Alcohol biomarkers can be used to measure the quantity of ethanol consumed and the time frame in which it was ingested. Such information can be vital in screening for excessive alcohol use among drivers or in workplace settings, as well as in monitoring abstinence and/or screening for relapse in alcohol dependence treatment (Helander et al., 2009). Potentially useful biomarkers most typically isolated from the blood and from the urine are summarised in Tables 1-6. The information provided in the columns headed ‘Drinking behaviour targeted’ and ‘Primary indication’ is determined based on the properties of the biomarkers, which are identified in the other data columns (‘Window of assessment’, ‘Sensitivity’, etc.). In other words, these properties determine the potential uses of the biomarkers, based on which they

are divided into three categories: 1) markers for chronic alcohol consumption; 2) trait markers for alcohol dependence; and 3) markers for acute alcohol consumption.

Table 1-6 *Alcohol Biomarkers in Blood*

Bio-marker*	**Drinking Behaviour Targeted**	**Window of Detection**	**Primary Indication**	**Sensitivity (percent)**	**Speci-ficity (per-cent)**
% CDT	Moderate to high (heavy drinking for 7 to 10 days)	2 to 3 weeks	Screening/ Relapse	Similar to GGT (26-83) (Stowell et al. 1997)	92
MCV	Chronic heavy drinking	Up to several months	Screening	Lower than GGT (47) (Anttila et al. 2004)	n/a
GGT	Chronic heavy drinking	2 to 3 weeks	Screening	Moderate (61) (Anttila et al., 2004)	n/a
AST and ALT	Chronic heavy drinking	2 to 3 weeks	Screening	Lower than GGT (AST 56) (Anttila et al., 2004) (<40)	n/a
FAEE	Unknown (at least several drinks)	Up to several months (2 days)	Screening/ Abstinence (Abstinence/ Relapse)	100 (Wurst et al., 2004)	90
PEth	Heavy drinking for ~5 days	1 to 2 weeks	Screening/ Relapse	99 (Aradottir et al., 2006)	–

*GGT, gamma-glutamyl transpeptidase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; MCV, mean corpuscular volume; CDT, carbohydrate-deficient transferrin; FAEE, fatty acid ethyl esters; PEth, phosphatidyl ethanol.

Alcohol biomarkers for chronic alcohol consumption

Carbohydrate-deficient transferrin (CDT) is an example of a marker known to have properties that make it useful in detecting chronic harmful intake of alcohol (Anton, 2001; Niemela, 2007). It is an iso-form of transferrin (a glycoprotein that is important in transportation of iron in plasma) that has two N-glycosylation sites (Landberg et al., 1995). Upon consumption of high alcohol levels for a long period, a shift in the carbohydrate composition in transferrin is observed and minor glycoforms (asialotransferrin and disialotransferrin) are detected. This shift remains detectable, and serum CDT levels take two to three weeks to return to normal after abstinence due to the long half-life of transferrin (1.5-2 weeks) (Jeppsson et al., 1993; Helander et al., 2001; Litten & Allen, 1998; Stibler, 1991). CDT is considered a more specific chronic heavy alcohol consumption biomarker when compared to other known biomarkers. This makes CDT suitable for use in detecting relapse (Anton et al., 2002; Bergstrom & Helander, 2008). However, its effective use requires that the participant has been drinking over a seven to 14 day period (Hannuksela et al., 2007).

Mean corpuscular volume (MCV) (i.e., red blood cell size) is another biomarker for chronic heavy alcohol consumption. Red blood cells typically increase in volume as an individual consumes more alcohol. This is due to a secondary reaction to bone marrow toxicity. However, MCV is considered a poor biomarker for acute alcohol consumption due to the fact that erythrocyte half-life is 120 days. Other factors can contribute to elevated MCV, moreover, so this measure is not sufficiently reliable to be used on its own and should routinely be used with aspartate aminotransferase (AST) to rule out delirium tremens (Conigrave et al., 2003; Findley et al., 2010; Niemela, 2007).

Gamma glutamyl transferase (GGT) is a membrane-bound glycoprotein enzyme that is an amino acid (Goldberg, 1980). It is a useful biomarker because its levels are known to be elevated after years of chronic alcohol consumption. A high level of GGT in the serum is associated with liver damage as it is highly detected in the liver, kidney, and bile. It is also increased in association with the use of certain prescription medications, such as anticonvulsants (Conigrave et al., 2002).

Finally, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), measured in connection with routine screening for liver diseases, are both typically elevated in cases of chronic alcohol consumption (Conigrave et al., 2002). AST is mitochondrial enzyme present mainly in the liver. However due to its presence in other organs, any factors affecting the muscles can lead to its elevation. ALT is more specific to liver damage as it is a cystosolic enzyme mainly present in the liver tissue (Conigrave et al., 2003).

Trait markers for alcohol dependence

This category consists of trait markers that can provide information relevant to whether a given individual might possess a genetic predisposition that could contribute to his or her susceptibility to alcohol dependence. These markers are associated with efforts to prevent rather than to screen for and/or treat excessive alcohol consumption or dependence (Hellevuo et al., 1997). They include Adenylyl Cyclase (AC) Activity, Gamma-Aminobutyric acid (GABA), Dopamine, Serotonin, and Beta-Endorphin (Hoffman et al., 2002; Oroszi & Goldman, 2004; Oswald & Wand 2004; Ratsma et al., 2002).

Alcohol biomarkers for acute alcohol consumption

Some markers that remain detectable for only a few days after alcohol use can be effective in detecting acute consumption within this timeframe. This class of markers includes serotonin metabolites 5-hydroxytryptophol (5-HTOL) and 5- hydroxyindole-3-acetic acid (5-HIAA). In urine, the ratio of 5-HTOL/5-HIAA increases following the intake of high levels of alcohol. This ratio has been found to be more sensitive than plasma ethanol levels and to be measurable for up to 20 hours after the disappearance of ethanol (Helander et al., 1992; Helander et al. 1993, 1996; Helander & Eriksson, 2002; Voltaire et al., 1992). Davis et al. (1967) documented that an interaction with the metabolism of serotonin (5- hydroxytryptamine, 5HT) results in a shift toward the production of 5HTOL during ethanol metabolism. This shift, which is represented in Figure 2-2, can be explained as due to processes that favour 5HTOL formation over the formation of 5HIAA. These processes include: 1) increased cytosolic NADH/NAD^+ ratio; 2) the inhibition of mitochondrial ALDH by acetaldehyde; and 3) increased cytosolic NADH/NAD^+ ratio (Svensson et al., 1999; Walsh, 1973). The ratio remains elevated for 6-20 hours after the disappearance of ethanol in plasma, which gives it an advantage over ethanol as a biomarker (Eriksson et al., 2002).

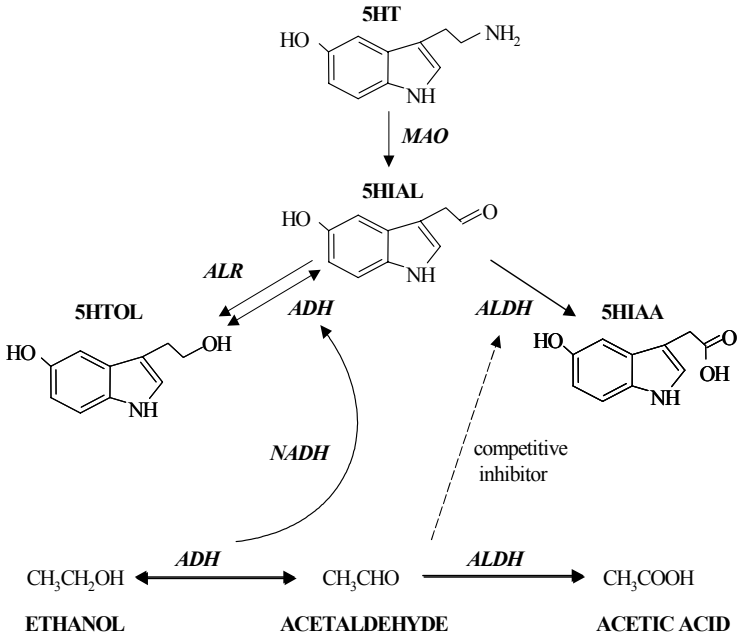


Figure 1-4 *Interaction between Ethanol and serotonin (5-HT).*

Other markers in this category include the products of non-oxidative ethanol metabolism, noted earlier. Of these, EtG (Wurst et al., 1999) and EtS (Helander & Beck, 2005) are both detectable in urine and remain so for up to several days after alcohol consumption (see Table 1-7). FAEE can be detected in blood for up to 24 hours, but in hair it can be detected for up to two months (Auwärter et al., 2001; Doyle et al., 1994; Pragst et al., 2001). Compared to EtG, however, concentrations of FAEE in hair samples have been found to correlate less reliably with volume of alcohol consumed (Auwärter et al., 2001; Süsse et al., 2010; Wurst et al., 2004).

Using Biomarkers in Combination

Many researchers believe that the most reliable approach to detecting and assessing alcohol consumption involves using two or more biomarkers in combination. The most common combination is CDT in conjunction with GGT (Litten et al., 1995).

Some investigators have developed mathematical equations for assessing CDT + GGT measurements. Sillanaukee and Olsson (2001), for example, provide the equation (0.8 x ln(GGT) + 1.3 x ln(CDT)). This approach was subsequently improved upon by replacing absolute CDT with %CDT (Anttila et al., 2003; Hietala et al., 2006).

Table 1-7 *Alcohol Biomarkers in Urine*

Biomarker*	Drinking Behaviour Targeted	Window of Detection	Primary Indication	Sensitivity
5-HTOL 5-HIAA	4 drinks	1 day	Screening/relapse	High
EtG	1 to 2 drinks (Unknown)	Several days (Up to several months)	Abstinence/relapse (Screening/abstinence)	Some false negatives (Unknown)
EtS	1 to 2 drinks	1 to 2 days	Abstinence/relapse	High

*HTOL, hydroxytryptophol; HIAA, hydroxyindoleacetic; EtG, ethyl glucuronide; EtS, ethyl sulfate.

1.2.4.2 Detecting EtG in Hair

EtG is highly acidic, non-volatile, non-oxidative, hydrophilic, and stable alcohol phase II metabolite. It was first detected in hair samples from individuals believed to have engaged in repeated alcohol consumption about two decades ago (Aderjan et al., 1994). Since that time, a number of researchers have proposed EtG in hair as a stable marker to detect and quantify alcohol consumption (Høiseth et al., 2007; Skopp et al., 2000; Wurst et al., 2003).

Since its initial application, numerous studies have investigated the reliability and merits of measuring EtG in hair samples taken from individuals with a history of alcohol dependence. Although many of these studies used only post-mortem samples,

others have investigated current alcohol dependent patients using this technique. Table 1-8 shows a summary of the results of selected studies of this kind.

Alt et al. (2000) investigated EtG in both alcohol dependents and social drinkers and found no EtG in samples from the latter group, whose members reported consuming about 20 g/day. Janda et al. (2002) detected EtG in 42% of samples from patients identified as alcohol dependent, compared to only one (EtG: 55pg/mg) out of the five samples collected from drinkers who consumed 30 g/day or less. However, the study did not report a correlation between the measured EtG and the reported levels of alcohol consumed. Similarly, Yegles et al. (2004) were successful in detecting EtG in alcohol dependent patients, but the authors indicated that there was no clear relationship between the EtG (or FAEE) concentrations and the self-reported alcohol consumption. Politi et al. (2006), however, reported EtG hair concentrations correlating with the daily alcohol consumption as assessed using a drinking questionnaire. Based on their results, the authors suggested cut-off values of 4 and 5 pg/mg to detect consumption of > 30 g alcohol/day and > 40 g alcohol/day, respectively.

Table 1-8 *Summary of Results of Selected Studies Measuring EtG Levels in Hair*

Study	**Number of Participants (n)**	**EtG (pg./mg)**	**Recommended range for cut-off values**
Alt et al. (2000)	Alcohol dependent (4) Social drinkers (6)	119-388 Nd	–
Janda et al. (2002)	Risk drinkers/alcohol dependent (60) Non-risk drinkers (5)	Nd-13157 Nd-55	–
Yegles et al. (2004)	Risk drinkers/alcohol dependent (10) Non-risk drinkers (4)	30–415 Nd	–
Politi et al. (2006)	Risk drinkers/alcohol dependent (22) Consuming 2 to 60 g/day alcohol (21)	4–434 Nd-35	*EDI>30g:4pg/mg EDI>40g:5pg/mg
Appenzeller et al. (2007)	Risk drinkers/alcohol dependent (15)	8–261	EDI 11–40 g: 4–15 pg/mg EDI > 60 g: 23 pg/mg
Kerekes et al. (2009) Kronstrand et al. (2012)	Risk + non-risk drinkers (32)	Nd-1146	EDI 16 g: < *SoHT, 7 pg/mg[a]
Kharbouche et al. (2012)	Consuming 32 g alcohol for 3 months Consuming 16 g alcohol for 3 months	Nd-11 Nd-3	EDI 32 g: < SoHT, 30 pg/mg[a] EDI>20g:9pg/mg
Stewart et al. (2012)	Risk drinkers (38) Non-risk drinkers (44)	Nd-1190 Nd-32	EDI> 60g:25pg/mg

*EDI = Ethanol Daily Intake; SoHT = Society of Hair Testing

In 2010 the Society of Hair Testing (SoHT) recommended cut-off levels to differentiate between excessive and social alcohol consumption (Albermann et al., 2011). The proposed cut-off for EtG in hair to strongly suggest chronic excessive alcohol consumption is 30 pg/mg scalp hair measured in the 0-3 to 0-6 cm proximal segment. If samples less than 3 cm are used, it is recommended that the results be interpreted cautiously. An EtG concentration equal to or greater than 7 pg/mg scalp hair indicates alcohol consumption and provides evidence to refute a claim of abstinence.

The SoHT cut-offs have been the participant of considerable debate. However, in a recent meta-analysis Boscolo-Berto et al. (2013) provided support for the SoHT thresholds, concluding that the cut-off of 30 pg/mg limits the false-negative effect in differentiating risk from non-risk drinkers, while the 7 pg/mg cut-off value constitutes sufficient evidence (only) for suspecting active alcohol use rather than complete abstinence.

Due in part to the presence of variables that can affect EtG levels in hair, however, some researchers propose cut-offs that are lower than the current standard. These include a proposed threshold for detecting 'moderate drinking' (defined in a number of studies as 28-40 g/day) of between 4 pg/mg and 15 pg/mg (Kharbouche et al., 2009; Politi et al., 2006; Stewart et al., 2013). Other studies propose cut-off values between 4 pg/mg (Politi et al., 2006) and 25 pg/mg (Kharbouche et al., 2012) for the identification of 'risk' drinking, often defined as consumption in excess of 40 g of alcohol per day.

Clearly, further research is needed in order to achieve consensus on reliable cut-off values for detecting alcohol use and for differentiating use from abuse. As the WHO

(2007) has indicated, however, this effort should take into account a wide set of criteria so that the interpretation of risk drinking reflects national, gender, and individual differences.

As noted above, less than 0.1% of ingested Ethanol is conjugated into EtG and EtS (Helander & Beck, 2004). Nevertheless, EtG and EtS remain detectable in urine for several days, giving these metabolites strong potential for use in screening for recent alcohol consumption. This section discusses some of the research findings regarding detection of these markers and the utility of information obtained from, beginning with EtG.

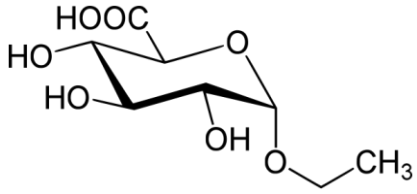


Figure 1-5 *Chemical structure of Ethyl glucoronide (EtG).*

EtG, the chemical structure of which is shown in Figure 1-5, is a metabolite of ethanol that is formed in small amounts by reaction with uridine-5-diphospho-β-glucuronic acid (UDPGA), which catalyses endoplasmic reticulum UDP-glucuronosyltransferase (UGT) enzymes (Tukey & Strassburg, 2001). Methods for measuring EtG have been developed based on gas chromatographic-mass spectrometric (GC-MS) (Wurst et al., 1999) and liquid chromatographic-mass spectrometric (LC-MS) techniques (Stephanson et al., 2002). Using these methods, EtG has been established as both a sensitive and a specific biomarker for the detection of recent alcohol intake (Wurst & Metzger, 2002). In fact, EtG has been found to be useful even in screening for low levels of alcohol consumption (7 g), and, at up to

four days, it has a relatively long window of detection in urine (Seidl et al., 2001; Stephanson et al., 2002).

Table 1-9 *Summary of Studies Investigating Urinary Ethyl Glucuronide (UEtG) Levels in Alcohol Dependent Patients*

Study	Population	n*	Results	Window of Detection
Alt et al. (1997)	Male patients in alcohol clinical study	33	UEtG 3.6–710 mg/l	Detectable up to 75 hours
Wurst et al. (1999)	Patients in alcohol withdrawal	33	UEtG 3.6–710 mg/l, No correlation of UEtG with SEC, GGT, MCV.	Detection 57.7 ± 16.9 hours
	Detoxified alcoholics	30	Four cases UEtG 4.2–196.6 mg/l, one case SEtG 4.8 mg/l	
	Neurorehabilitation patients	43	Eight urine samples from seven patients positive UEtG 2.9–23.49 mg/l	
Wurst et al. (2000)	Detoxified polydrug-abusing patients	9	Four cases positive	
	Detoxified alcoholics	24	UEtG 0.29–1.03 mg/l	
Wurst et al. (2003)	Forensic psychiatric in-patients with substance dependence	35	During 1 year: 14 of 146 urine samples positive for EtG Other biomarkers giving an indication for lapse/relapse (breath ethanol, CDT, GGT, MCV, phosphatidyl ethanol)	

*n = number of participants in the study.

Table 1-9 provides a summary of results of selected studies that have investigated the levels of urinary EtG (UEtG) in alcohol dependent patients. One of the earliest studies of this kind was that of Alt et al. (1997), who found UEtG levels between 3.6 and 710

mg/L and, further, determined that detection remained possible for up to 75 hours. Later, Wurst et al. (2009) detected UEtG up to 80 hours after consumption in alcohol dependent patients undergoing detoxification. However, the detection window has been noted as highly variable across individuals. Wurst et al. 2009 found variability in the detection window from 40 to 130 hours for UEtG and from 55 and 110 hours for UEtS. Similarly, Helander et al. (2009) found that after 30 to 110 hours the presence of EtG resulting from alcohol intake was no longer detectable, as levels dropped to below the cut-off of <500 ng/mL.

Although concentrations of EtS in urine (UEtS levels) after alcohol consumption are typically lower than those for EtG, increased attention has recently been focused on the potential of EtS as a biochemical marker for acute alcohol intake (Dresen et al., 2004), and Helander & Beck (2004) have been successful in measuring its concentrations using LC-MS. Helander et al. (2009) investigated the levels of both EtG and EtS in urine samples from alcohol dependent patients undergoing detoxification. The authors also surveyed previous findings regarding the detection time frame for EtG after alcohol intake. Table 1-10 summarizes some of these results.

Table 1-10 presents studies conducted using healthy volunteers as well as alcohol dependent patients or clients receiving drug treatment. The results of these studies indicate that increased levels of UEtG are commonly detectable between 24 and 48 hours after alcohol consumption in healthy participants, compared to patients undergoing alcohol detoxification, for whom the window was found to extend up to 80 hours (Dahl et al., 2002; Wurst et al., 1999).

Table 1-10 *Results of Selected Studies Investigating the Measurement of UEtG and UEtS (Helander et al., 2009)*

			Detection Window (h)		*Cut-off Limit (mg/L)*		*Major Results*	*Study*
Population	**n***	**Alcohol Dose (g/kg of body weight)**	**EtG**	**EtS**	**EtG**	**EtS**		
Healthy participants	4	**0.1** (0.33 L of low-alcohol beer (2.2% ethanol, w/w) in 15–30 minutes))	≤6	–	0.1	–	Intake of very small amount of ethanol (7 g) produced ethyl glucuronide values up to 8.4 mg/L after 4 hours	Stephanson et al. (2002)
	2	**0.1**	13-22	22-26	0.15	0.11		Wurst et al. (2006)
	9	**0.15**	–	≤12	0.1	–		Helander and Beck (2005)
	5	**0.25**	<24	–	0.1	–		Wojcik and Hawthorne (2007)
	6	**0.2–0.3** 0.2 g/kg ethanol (0.2, 0.1–0.33, 0.07) over 20–30 minutes	3–25	5-30	0.15	0.11		Wurst et al. (2006)
	6	**0.5** (0.5 g/kg (range25.0-41.5 g) as5% (v/v) beer in 30 min)	22-32	–	0.1	–	About 0.02% of the ingested ethanol dose (on a molar basis) was recovered in the urine as EtG	Dahl et al. (2002)
	10	**0.5**	25-35	–	0.2	–		Hoiseth et al., (2007)
	9	**0.5**	–	≤24	0.1	–		Helander and Beck (2005)
	1	**0.5**	≤29	≤29	0.1	0.1		Helander and Beck (2004)
	10	**0.5**	25-48	25-39	0.1	0.1		Hoiseth et al. (2008)
	7	**0.5**	≤48	–	0.1	–		Wojcik and Hawthorne (2007)
	1	**0.6**	≤36	≤36	0.15	0.11		Wurst et al. (2006)
	13	**0.50–0.78**	27-44	23-47	0.1	0.1		Halter et al. (2008)
	7	**0.75–0.85**	≤48	–	0.1	–		Wojcik and Hawthorne (2007)
	17	**>1**	39-102	–	0.1	–		Borucki et al. (2005)

More recently, Stewart et al. (2013) investigated the sensitivity and specificity of the information provided by both EtG and EtS in urine samples. The results indicated that UEtG (sensitivity 76%, specificity 93%) and UEtS (sensitivity 82%, specificity 86%) both performed well in identifying recent drinking, and that the performance of these biomarkers appeared to be unaffected by the presence or absence of liver disease.

Although consensus may still be some way off, establishing standard cut-off limits and windows of detection for EtG and EtS screening is an important goal, as such standards can provide conformity across application contexts, including those with legal implications (e.g., motor vehicle operator screening) and work place drug testing (WDT), as well as clinical diagnosis and treatment (Helander et al., 2009). In 2012 the Substance Abuse and Mental Health Services Administration (SAMHSA) published an update to its 2006 Advisory Report that included the following recommended terminology and cut-offs for use in screening for alcohol use by means of UEtG and UEtS: A 'high' positive (e.g.,>1,000ng/mL) may indicate: heavy drinking on the same day or previously (e.g., previous day or two); light drinking the same day. A 'low' positive (e.g., 500-1,000ng/mL) may indicate: previous heavy drinking (previous 1–3 days); recent light drinking (e.g., past 24 hours); or recent intense 'extraneous exposure' (within 24 hours or less). A 'very low' positive (100-500ng/mL) may indicate: previous heavy drinking (1–3 days); previous light drinking (12–36 hours); or recent 'extraneous exposure') (SAMHSA, 2012).

Finally, in forensic settings in which a 'preponderance of the evidence' is sought to establish 'proof of drinking', Skipper et al. (2004) recommend the following guidelines: Cut-offs: Utilize a cut-off or threshold of at least 500ng/ml for EtG and 100ng/ml for EtS (using values normalized to creatinine 100mg/dl) for 'proof of

drinking.' It is acceptable to have a lower 'reporting cut-off'; however, levels between 100-500ng/ml are statistically more likely to be from extraneous, non-beverage sources of alcohol (SAMHSA, 2012).

Alcohol consumption is associated with problems in a variety of populations, and the availability of reliable, broadly applicable measurement and monitoring mechanisms can have a tremendous positive impact on the success of research, clinical interventions, and monitoring in a variety of settings. In this regard, the biomarkers EtG and EtS appear to be potentially highly useful, as they possess sufficient sensitivity and duration for use in monitoring for relapse in clinical settings – which can be vital to ensuring desired treatment outcomes – as well as for use in WDT with populations critical to public safety, such as pilots, air traffic controllers, and medical personnel. Indeed, Wurst (2009) found that the use of these markers could reveal acute alcohol consumption in individuals who score below levels indicating 'harmful drinking' on a self-reported measure (AUDIT). Their value in screening in contexts that require direct and reliable measures of alcohol use should therefore not be underestimated. Self-reported measures, however, can still provide a more broadly descriptive picture of alcohol use and related behaviours than biomarkers alone. Therefore, in clinical and research settings, an approach that combines both types of tool should be considered the most appropriate.

1.3 Alcohol Use During Methadone Treatment and Potential Interaction

1.3.1 Introduction

A nationwide co-morbidity survey conducted in the United States estimated that 7% of the total population had developed a dependence on an illicit drug at some point in their lives (Anthony et al., 1994). Not long after, the number of drug users presenting for treatment in England was estimated to be 24,000, with an estimated 30,000 in the UK as a whole (Central Drugs Coordinating Unit, 1998). Clients presenting for treatment are often multiple drug users (Gossop, 2001), and multiple drug use presents an additional challenge for drug treatment units that has a considerable impact on both treatment outcomes and public health issues. Multiple drug users usually present with critical medical and psychiatric conditions such as HIV-AIDS (acquired immune deficiency syndrome), liver cirrhosis, and major depression; in many cases, multiple medications are prescribed, and this can result in drug-drug interactions (Kapur et al., 2011).

In the UK, 3,604 deaths linked to methadone were recorded between 1993 and 2002, but methadone was not necessarily the cause of death in all cases (Corkery et al. 2004). According to the WHO (2009), drug interactions are a factor in both morbidity and mortality, and this includes cases involving patients receiving methadone treatment. In the United States, 552 deaths were reported as related to methadone in 1996, and they occurred during the first weeks of starting methadone treatment (Karch & Stephens, 2000). Butler et al. (2011) investigated fatalities linked to methadone and arrhythmias in MMT and found that out of 30 deaths due to toxicity only two cases were linked to arrhythmias. Results were also consistent with previous

studies, which indicated that 75% of deaths happened during maintenance (Butler et al.).

With respect to cases of alcohol interaction in methadone overdose, Ruttenber et al. (1990) investigated 505 fatal heroin overdoses and found that the correlation between blood alcohol levels and opiate concentrations was significantly negative, meaning that a significant inverse correlation was found between blood alcohol and morphine levels (Ruttenber et al.). In other words, it appears that in the presence of alcohol a smaller amount of opiate/opioid can lead to a fatal overdose. The co-dependency of alcohol and illicit drugs, moreover, appears to be high and is also present in patients receiving MMT (Srivastava et al., 2008). This chapter therefore reviews the relationship between alcohol and methadone and highlights the importance of managing clients who present with alcohol problems when receiving methadone treatment.

1.3.2 Alcohol consumption during methadone maintenance treatment

The prevalence of alcohol consumption in patients receiving MMT has been well documented in the literature. Srivastava et al. (2008) conducted a review to determine whether alcohol consumption is affected during the course of MMT. The review identified fifteen clinical studies, of which 11 were conducted in the United States. Most were cohort studies, except for three that were randomised control trials (Schottenfeld et al., 1998; Strain et al., 1996; Strang et al., 2000). The patient populations varied, but the majority were recruited from methadone maintenance; sample size varied between 40 and 625 participants. During the MMT, three studies

observed an increase in alcohol use, three observed a decrease in alcohol use, and nine observed no change in alcohol use. The review found that alcohol use, although often problematic in methadone-using patients, is not likely to change upon entering MMT. The authors concluded, however, that although there is no significant change in alcohol consumption upon starting MMT, the stability of alcohol consumption should still be considered problematic, and that it is important to prioritise addressing problematic alcohol consumption in MMT (Srivastava et al.). Nonetheless, the appropriate management of alcohol dependent patients undergoing MMT has not been substantially addressed (Hillebrand et al., 2001).

Some patients develop alcohol problems after they start methadone treatment. Alcohol in this case may serve as a substitute for heroin and may be used to self-medicate in response to withdrawal symptoms in cases where the patient perceives methadone doses as insufficient (Gordis & Sereny, 1980). One of the largest cohort studies of drug misusers in the United Kingdom, The National Treatment Outcome Research Study (Gossop et al., 2000), looked at multisite treatment outcomes of 1,075 drug users who were recruited from MMT and methadone reduction treatment programs. The study found a high prevalence of the use of illicit drugs among the clients as well as heavy alcohol consumption. A one-year follow-up study found a reduction in the use of illicit drugs, but the pattern of alcohol consumption remained the same (Gossop et al.). A later paper investigated alcohol outcomes and heavy drinking in 418 of the drug misusers from the NTORS study after four to five years and found that heavy drinking prior to treatment was a strong predictor of heavy drinking after treatment. The study also found that only a minority of the clients exhibiting the sample pattern of drinking was using alcohol as a substitute for opioids (Gossop et al., 2003).

Complications due to problematic alcohol consumption during MMT can include negative treatment outcomes, interaction between alcohol and methadone leading to increased medical complications – including the risk of death by overdose – and unstable therapeutic drug levels, which can cause patient non-compliance (Staiger et al., 2013). Stenbacka et al. (2007) conducted a cohort study and recruited 204 opioid dependent patients who were admitted to receive MMT. The patients were followed up between 1995 and 2000 to analyse the frequency of problematic alcohol consumption and the effect of their problematic drinking (both prior to and during MMT) on retention in treatment. Alcohol use was measured using biomarkers, including 5-hydroxytryptophol to 5-hydroxyindoleacetic acid ratio (5HTOL/5HIAA) (to indicate recent alcohol consumption), as well as any records of alcohol dependency treatment and any history of inpatient care. Only 96 participants were tested for urinary biomarkers, and about half (n=44) tested positive (> 15nmol/umol) at least once during the follow up. During the study about 12% of the participants were treated for alcohol related problems. The risk of relapse seemed to peak within four months after starting the treatment. The study also found an increase in relapse and early discharge in patients with related problematic drinking (Stenbacka et al.).

1.3.3 Clinical and pharmacological considerations in alcohol and methadone co-dependence

Alcohol and methadone have similar mechanisms of action leading to the depressant effects on the Central Nervous System (CNS). This could be a contributing factor in the adverse consequences of concurrent alcohol and methadone use, such as an overdose due to drug-drug interaction, which has been mainly acknowledged as a pharmacodynamic interaction (Best & Ridge, 2003). However, an overview of the

metabolic relationship between alcohol and methadone demonstrates a pharmacokinetic interaction that may negatively affect treatment outcomes.

Depending on the amount and duration, alcohol consumption can influence hepatic metabolism, which in the case of patients receiving methadone maintenance treatment can affect the pharmacokinetics of methadone (Clark et al., 2006). Short-term consumption leads to a competitive inhibition of the CYP2E1 enzyme, whereas chronic use leads to its induction. CYP2E1 induction affects the metabolism of some drugs that an alcohol dependent patient may be consuming. For example, an increase in the clearance of warfarin, diazepam, rifampycin, pentobarbital, and alcohol itself occurs. This induction may lead to problematic interactions with other drugs when taken concomitantly. Chronic alcohol consumption, however, has been reported to induce production of the liver enzyme Cytochrome P450 2E1 – a key enzyme in the microsomal enzyme ethanol-oxidizing system (MEOS). When activated, it can lead to a 50% increase in alcohol metabolism. CYP450 2E1 induction causes an increase of alcohol elimination.

In patients who are chronic alcohol consumers receiving methadone, hepatic pathophysiological changes can indirectly affect the metabolism of methadone and reduce the peak methadone concentration (Clark et al., 2006). Alcohol induces the activity of CYP3A4, causing drugs metabolised by this enzyme to decrease, including methadone (Klotz & Ammon, 1998; Kreek et al., 1980). Methadone is metabolised by CYP3A4; therefore, the induction of the enzyme leads to a reduction in methadone and ethanol levels. This may explain why some patients find that their daily methadone dose does not remain effective for 24 hours and may need their dose to be

administered twice instead of once daily (Dyer & White, 1997).

Unlike chronic alcohol consumption, acute alcohol consumption increases the peak methadone concentration. This is due to the different pathway by which alcohol is metabolised when consumed in high quantities over a short period of time. Acute consumption of alcohol can compete with methadone for the CYP3A4 enzyme. This can explain the higher methadone and alcohol levels in this patient population (Clark et al., 2006).

The change in the rate of metabolism can directly affect other pharmacokinetic parameters, including the half-life and elimination time for both alcohol and methadone. Wolff et al. (2000) looked at the benefits of plasma methadone measurements in dosage adjustment during MMT and discussed the use of the methadone plasma levels to help with the clinical assessment of withdrawal symptoms since there has been evidence to indicate a correlation between methadone levels and participantive symptoms of withdrawal. However, the concomitant use of alcohol can be dangerous, as it can lead to drug-drug interaction affecting plasma methadone levels (Wolff et al., 1997).

Although most of the literature addresses the effects of alcohol on methadone levels, Clark et al. (2006) assessed the impact of opioid substitution therapy on alcohol metabolism. Forty patients receiving opioid substitution therapy were given alcohol in two sessions. One session was pre-opioid pharmacotherapy and the second session was post-opioid therapy. The results indicated an interaction between ethanol and opioids after the administration of the opioid dose. Evidence of a dose-response relationship suggested that the blood alcohol concentration in opioid users was more

evident after the pre-opioid dose. The authors suggested that the interaction could be through microsomal enzymes and/or hormonal interactions (Clark et al.).

El-Bassel et al. (1993) investigated predictors of alcohol dependence among 201 patients in three MMT centres. The study found that metabolic processes in patients undergoing alcohol detoxification while being maintained on methadone could also be affected by previous hepatic pathophysiological changes caused by alcohol consumption, and that further adjustment of the methadone dose could be necessary to avoid distressing withdrawal symptoms in patients receiving MMT. Therefore, alcohol dependent patients receiving MMT might need different doses of methadone than patients who are not alcohol dependent (El-Bassel et al.).

The complicated overlap between methadone and alcohol makes it challenging to fully differentiate the causes of withdrawal symptoms in dual dependent patients. Although a few studies have suggested that methadone plasma concentrations can be monitored to adjust treatment dosage (e.g., Wolff et al., 1997), measuring the withdrawal symptoms could be an indirect way to monitor the loss of the drug from the body, and this method has been used to assess the effectiveness of pharmacotherapy. Nonetheless, patients presenting with dual dependencies are more challenging to treat due to the potential that withdrawal symptoms from the two dependencies, as well as the effects of administered drugs such as benzodiazepine, could overlap and exacerbate withdrawal symptoms (de Wet et al., 2004).

All in all, definitive knowledge exists regarding the link between alcohol and methadone pharmacodynamic interaction. Pharmacokinetic interaction has also been investigated in literature. However, a clear understanding of the mechanism by which

CYP3A4 contributes to this interaction remains unclear. Furthermore, the effects of alcohol on the ratio of EDDP/methadone have not been investigated.

1.3.4 Illicit drug use during methadone treatment

Methadone, when used in combination with certain drugs with depressant effects, such as alcohol and benzodiazepines, can result in an augmented action leading to an increase in its respiratory depressant effect and, in some cases, contributing to death (Caplehorn & Drummer 2002). In a study in New South Wales that examined 329 heroin users, of those who overdosed, around 79% had consumed another drug around the time of overdose, and alcohol, benzodiazepines, and opiates were the most commonly used (Darke, Ross et al. 1996).

Further studies investigating therapeutic plasma concentrations of methadone have indicated an overlap between therapeutic and fatal concentrations. For example, to achieve sufficient control of withdrawal symptoms required between 150–200 μg/L, and later during maintenance concentrations needed to be above 400 μg/L (Loimer et al., 1992). However, Worm et al. (1993) investigated cases of methadone-related deaths, which they divided depending on the level of alcohol present in the blood (>50 mg/100 ml). Methadone blood concentration was found to be 60–3090 μg/L (median, 280; mean, 430) in 59 cases with no alcohol. However, methadone concentrations in eight of the cases in which alcohol was detected ranged between 90–650 μg/litre (median, 150; mean, 250). The study also investigated methadone concentrations in patients receiving methadone maintenance; in 62 patients methadone concentration was 30–560 μg/litre (median, 110; mean, 140), compared to methadone concentrations of 30–900 μg/litre (median, 90; mean, 150) in patients with alcohol present (Worm et al.) (see Table 1-11).

Table 1-11 *Methadone Concentrations in Patient with or without Alcohol (Worm et al., 1993)*

Number of patients (n)	Alcohol Detection	Methadone concentration
59	No alcohol	60–3090 µg/L
8	Alcohol detected	90–650 µg/L
62	No alcohol	30–560 µg/L
35	Alcohol detected	30–900 µg/L

The toxicological report therefore confirms the overlap between clinical therapeutic concentrations and lethal concentrations, a finding that needs to be further investigated in order to avoid the overestimation of direct methadone-related deaths. Also, studies are yet to establish the levels of alcohol in blood in combination with methadone that can be fatal. In practice, guidelines recommend screening for alcohol using the breathalysers and drunk driving cut-offs, so that patients presenting with intoxication are not dispensed their daily dose of methadone to avoid concomitant effects of alcohol and methadone.

1.3.5 Clinical guidelines for monitoring alcohol use during methadone treatment

Clinical guidelines specifically address the lack of research in managing clients receiving methadone treatment who have concurrent alcohol problems. They recommend that risk assessments be undertaken in order to weigh the benefits of continuing methadone treatment. The National Treatment Agency for Drug Misuse (*Towards Successful Treatment Completion: Good Practice Guide*) addresses in detail the management of clients presenting with alcohol problems. The guidelines recommend that clinicians working with drug misusers be required to have ‘An awareness that alcohol misuse needs to be addressed alongside the management of

misuse of other drugs, competence at detecting problem drinking, an ability to give harm reduction and educational messages regarding misuse of alcohol, [and] competence to be able to manage alcohol misuse in drug misusers, including pharmacotherapies such as substitute prescribing.' The guide also notes that 'drug misusers who are dependent on alcohol should be offered alcohol interventions' (NTA, 2010). Regarding the use of breathalysers, the recommendation is as follows:

> If a client is breathalysed and the breath alcohol level is found to be above the legal drink-drive limit, they should be advised not to drive. Further guidance on the regulations relating to driving under the influence of drugs or alcohol is available in the Driver and Vehicle Licensing Agency's (DVLA) At a Glance Guide (DVLA, 2007) and also in appendix A7 of the Drug Misuse and Dependence: UK guidelines on clinical management (Department of Health and devolved administrations, 2007) (p.28).

The guidelines also report on an audit that investigated the use of breathalysers among 15 local practices in London. The audit indicated the following:

> Some services use breathalysers in an attempt to quantify the level of alcohol intoxication at the time of presentation and use this as a guide in the decision making process as to whether to dispense medication or not, sometimes refusing medication when the breathalyser reading is above a pre-determined point. In the audit all services stated a level of breath alcohol concentration above which they would not usually dispense or prescribe methadone – this limit ranged from 0 to 0.4mg/L, with most quoting the drink driving limit of 0.35mg/L. Six out of 15 services said they would dispense above this level in certain clinical scenarios, such as high levels of alcohol dependence and a client presenting in a state of alcohol withdrawal despite being above the drink driving limit.

Finally, the guidelines summarise best practices in managing alcohol intoxication or

dependence among clients receiving methadone treatment during supervised consumption (see Table 1-12). The guidelines highlight that the practice of using breathalysers to monitor alcohol intoxication supervised consumption is not routine in all clinical settings and that the alcohol drink driving limit is not always applied, with no scientific justification for the choice of these levels.

Table 1-12 *Guidelines for good practice in managing alcohol or benzodiazepine misuse in addition to an opioid prescripton (adapted fom the NTA 'Towards successful treatment completion: a good practice guide', 2009)*

Problems	Options
Client using alcohol/benzodiazepines to get intoxicated	Risk assessment; increase key working; add psychosocial interventions; change to supervised consumption of opioid prescription; regular breathalyser testing
Client dependent on alcohol/benzodiazepines	Risk assessment; alcohol/benzodiazepine community or inpatient medically assisted withdrawal regimen; increased key working; add psychosocial interventions; change to supervised consumption of opioid prescription; regular breathalyser testing; conduct health assessment and reflect finding back to client; consider inpatient detoxification leading to residential rehabilitation

1.3.6 Biological tools

1.3.6.1 Alcohol screening using breathalysers during methadone maintenance

Studies of alcohol consumption indicate that 0.7% of the ethanol consumed is excreted through breath (Ramchandani et al., 2001). It has also been shown that the

concentration of alcohol in blood is 2,448 times the concentration of alcohol in expelled air (Jones & Andersson, 2003). This relationship allows an individual's blood alcohol concentration (BAC) to be measured indirectly, via the concentration of alcohol in his or her breath (BrAC). Although most countries report BAC as a mass/volume ratio, Germany and the Nordic countries report it as a mass/mass unit (Jones et al., 2010). In either case, the level of intoxication can be determined by the concentration. Table 1-13 presents the stages of alcohol intoxication according to NIAAA (1994).

The *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V)* (American Psychiatric Association, 1994) lists behaviours that define intoxication, including recent ingestion of alcohol, clinically significant problematic behavioral or psychological changes (e.g., inappropriate sexual or aggressive behavior, mood liability, and impaired judgment) that developed during, or shortly after, alcohol ingestion, One (or more) of the following signs or symptoms developing during, or shortly after, alcohol use: (slurred speech, incoordination, unsteady gait, nystagmus, impairment in attention or memory, stupor or coma), and the signs or symptoms are not attributable to another medical condition and are not better explained by another mental disorder, including intoxication with another substance. From the definition of intoxication and the stages mentioned above, it is clear what challenges specialists could face in seeking to identify intoxication due to the many contributing factors.

Table 1-13 *Stage of Alcohol Intoxication (adapted from NIAAA, 1994)*

BAC (g/100 ml of blood or g/210 l of breath)	**Stage**	**Clinical symptoms**
0.01 - 0.05	Subclinical	Behaviour nearly normal by ordinary observation
0.03 - 0.12	Euphoria	Mild euphoria, sociability, talkativeness; increased self-confidence; decreased inhibitions; diminution of attention, judgment and control; beginning of sensory-motor impairment; loss of efficiency in finer performance tests
0.09 - 0.25	Excitement	Emotional instability; loss of critical judgment; impairment of perception, memory and comprehension; decreased sensory response; increased reaction time; reduced visual acuity; peripheral vision and glare recovery; sensory-motor incoordination; impaired balance; drowsiness
0.18 - 0.30	Confusion	Disorientation, mental confusion; dizziness; exaggerated emotional states; disturbances of vision and of perception of color, form, motion and dimensions
		Increased pain threshold; increased muscular incoordination; staggering gait; slurred speech; apathy; lethargy
0.25 - 0.40	Stupor	General inertia; approaching loss of motor functions; markedly decreased response to stimuli; marked muscular incoordination; inability to stand or walk vomiting; incontinence
		Impaired consciousness; sleep or stupor
0.35 - 0.50	Coma	Complete unconsciousness; depressed or abolished reflexes; subnormal body temperature; incontinence
		Impairment of circulation and respiration; possible death
0.45 +	Death	Death from respiratory arrest

During methadone dispensing, identifying intoxication is important alongside identifying and managing patients presenting with alcohol problems. The breathalyser has been used in some settings, adopting roadside cut-off limits to identify intoxication. In roadside testing, it was not until 1967 that a defined measure of alcohol limit (80 mg/100 ml) was implemented as part of the Road Safety Act in the UK (British Medical Journal, 1967). It has remained unchanged since then. Alcohol screening using a breathalyser is also used in emergency care settings.

Breathalysers are used in MMT, both for research purposes (Bickel et al., 1989; Helander et al., 1999; Marcovici et al., 1980) and during supervised consumption to screen for recent alcohol intake. One of the first studies to use breathalysers in a research setting among methadone maintenance patients was that of Marcovici et al., which investigated the relationship between methadone and problematic alcohol consumption in 60 male drug dependents starting methadone maintenance. The breathalyser was used as a tool to monitor participants' drinking, and results indicated that stabilization was not etiologically associated with alcohol abuse (Marcovici et al.).

A more recent study by Clark et al. (2006) used breathalysers to reflect methadone metabolism, investigating whether there was a dose-dependent effect of opioid substitution therapy on BAC by comparing BAC resulting from a standard dose of alcohol before pharmacotherapy with that after pharmacotherapy dose administration. The study also examined whether regular consumption of methadone, LAAM, or buprenorphine resulted in differential effects on BAC before/after dosing in opioid substitution therapy. The authors recruited forty opioid substitution patients, with 14 receiving methadone, 14 receiving LAAM, and 12 receiving buprenorphine. All

participants received 14.7 g/70 kg of alcohol and the BAC was later measured using a LION SD-2 breathalyser at 30, 55, and 80 minutes. Results indicated that there was an evident effect of the opioid on the alcohol levels in the first 1–2 hours after the opioid dose, leading to a reduction in BAC in the opioid users compared to the pre-opioid dose. The results also indicated that although the BAC in the control group was higher than in the patients receiving opioids, the difference was not significant (Clark et al. 2006).

Helander et al. (1999) compared the efficacy of using breathalysers to testing the urinary 5HTOL/5HIAA ratio as a biomarker of drinking. The study aimed to estimate the incidence of current alcohol consumption among methadone maintained outpatients. Results indicated that breathalyser tests were positive in only four of the 177 participants who reported alcohol use the previous day, which ranged between 10–230 g. However, 17 participants had positive urinary 5HTOL/5HIAA ratios (Helander et al.).

Table 1-14 *Studies that have used breathalysers in methadone maintenance*

Title	Objective	Breath-alyser	Cut-off limit	Results	Author, Year
Risk for Alcoholism United and Methadone States Treatment. A Longitudinal study	MAST, breathalyser, laboratory values and interviews, and NCA criteria were all used to categorize drinkers.	Not specified	Not specified	No significant changes were observed in alcohol consumption in problem or normal drinkers after 6 months on MMT	Marcovici et al., 1980
Comparison of urinary 5-hydroxytryptophol, breath ethanol, and self-report for detection of recent alcohol use during outpatient treatment: a study on methadone patients	To compare the results of breath-ethanol testing and a sensitive biochemical marker of recent drinking, the urinary 5HTOL/5HIAA ratio, with the results of a confidential self-report questionnaire, and, furthermore, to estimate the incidence of current alcohol consumption among methadone-maintained outpatients.	Alcolmeter S-D2	The detection limit of the method is ~0.01 g/l (~ 0.22 mmol/l). The breath-ethanol device was tested and calibrated by the manufacturer's local representative once every third month.	59 of 190 methadone-maintained outpatients (31.1%) had been drinking alcohol on the previous day according to self-report data and the results of urinary 5HTOL/ 5HIAA testing, but only four identified by utilizing breath-ethanol tests.	Helander et al., 1999

To the present author's knowledge, no study has directly investigated the use of the breathalyser as a tool in methadone maintenance supervised consumption to screen for alcohol use using the drunk driving limit. However, research investigating the impact of dual use of alcohol and methadone has been well established in linking the concomitant effects that can lead to fatal overdose.

Furthermore, alcohol screening using breathalyser has been linked with road safety and it was in 1967 that a defined measure of alcohol limit of 80 mg/ 100 ml using breathalysers was implemented as part of the Road Safety Act in the UK and remains the unchanged since then. A further understanding on how methadone can influence driving can help in understanding the rationale behind using brethlysers in monitoring patients presenting to methadone maintenance clinics. The following section reviews the relationship between methadone and driving prevalence, risks and applicable regulations.

Chapter 2 STUDY DESCRIPTION

This chapter describes the rationale and purpose underlying this thesis. It explains the overall study design, including the criteria used for the selection of participants, data collection procedures, and measures employed. The chapter also outlines both the laboratory analysis procedures and the statistical analyses that were conducted on the samples, as well as describing the research methods employed in the three studies.

2.1 Rationale

Methadone is acknowledged as an effective pharmacological substitution treatment for heroin dependence. However, patients presenting to addiction treatment services usually have multiple substance misuse issues, as well as both mental and physical health and social problems. Excessive alcohol use in individuals receiving MMT is well established as a challenge to clinical treatment.

While methods for managing single drug dependence are well established, the treatment of co-dependents, i.e., patients who are receiving MMT who also present with problematic alcohol use, has not been fully investigated. Therefore, further research is needed in order to explore the efficacy of current management techniques for patients with dual dependence. Research of this kind can help to develop new guidelines that are tailored to the complex dual-dependent population and thus lead to better treatment outcomes. This thesis seeks to explore ways in which to help clinicians improve the treatment outcomes for this group of patients by investigating biological tools that can be used to monitor the treatment process.

In current clinical practice, treatment outcomes tend to be assessed in terms of patient retention and cessation of the use of illicit drugs while in treatment. While these aspects of any pharmacotherapeutic programme are valuable, they do not provide an objective

indication of the efficacy or efficiency of methadone as a pharmacological agent. The aim of this research was to investigate biological markers that can be used to monitor methadone treatment and to determine whether their use can be beneficial to clinical treatment outcomes.

2.2 Purpose of the study

The aim of this threefold study was to investigate the potential for utilising biological tools with clients receiving MMT as an objective measure of the efficacy and efficiency of methadone treatment.

2.3 Research Questions (Premise of the Studies)

The research questions or premises that led to the experiments conducted for the study, and hence to the results and discussion presented in the chapters below, were as follows:

1. A study to explore the relationship of methadone with its primary metabolite EDDP and to determine whether the EDDP:methadone ratio is useful as a biomarker in assessing compliance among patients receiving methadone treatment (Study 1)
    a. A secondary study to explore the impact of hazardous alcohol use on the EDDP:methadone ratio compared to a methadone control group
    b. A study to explore the relationship of methadone and its primary metabolite and determine if the EDDP:methadone ratio changes during the induction period (Study 1.b)
2. A study to explore whether the urinary alcohol metabolites Ethyl glucuronide (EtG) and ethyl sulphate (EtS) are useful in identifying recent alcohol consumption during methadone treatment (Study 2)

3. A study to explore the assessment of recent alcohol consumption using the breathalyser test and to determine whether the established cut-off level is appropriate as a tool for clinical decisions regarding the dispensing of methadone (Study 3)

2.4 Null Hypotheses

1. There will be no variation in the EDDP:methadone ratio among patients receiving methadone treatment, irrespective of the daily methadone dose prescribed. (Study 1)

2. The urinary alcohol metabolites EtG and EtS are not useful in identifying recent alcohol consumption during MMT. (Study 2)

3. The breathalyser test is not useful as a clinical guide for decisions regarding the prescribing and dispensing of methadone. (Study 3)

Chapter 3 METHODOLOGY

3.1 The research environment

The research for this thesis was carried out in an area of approximately 11 square miles in South East London. Much of this region is taken up by the London Borough of Southwark, which is the second largest inner borough in London. It is divided into four localities:

- Bermondsey & Rotherhithe
- Borough & Walworth
- Peckham & Camberwell
- Dulwich

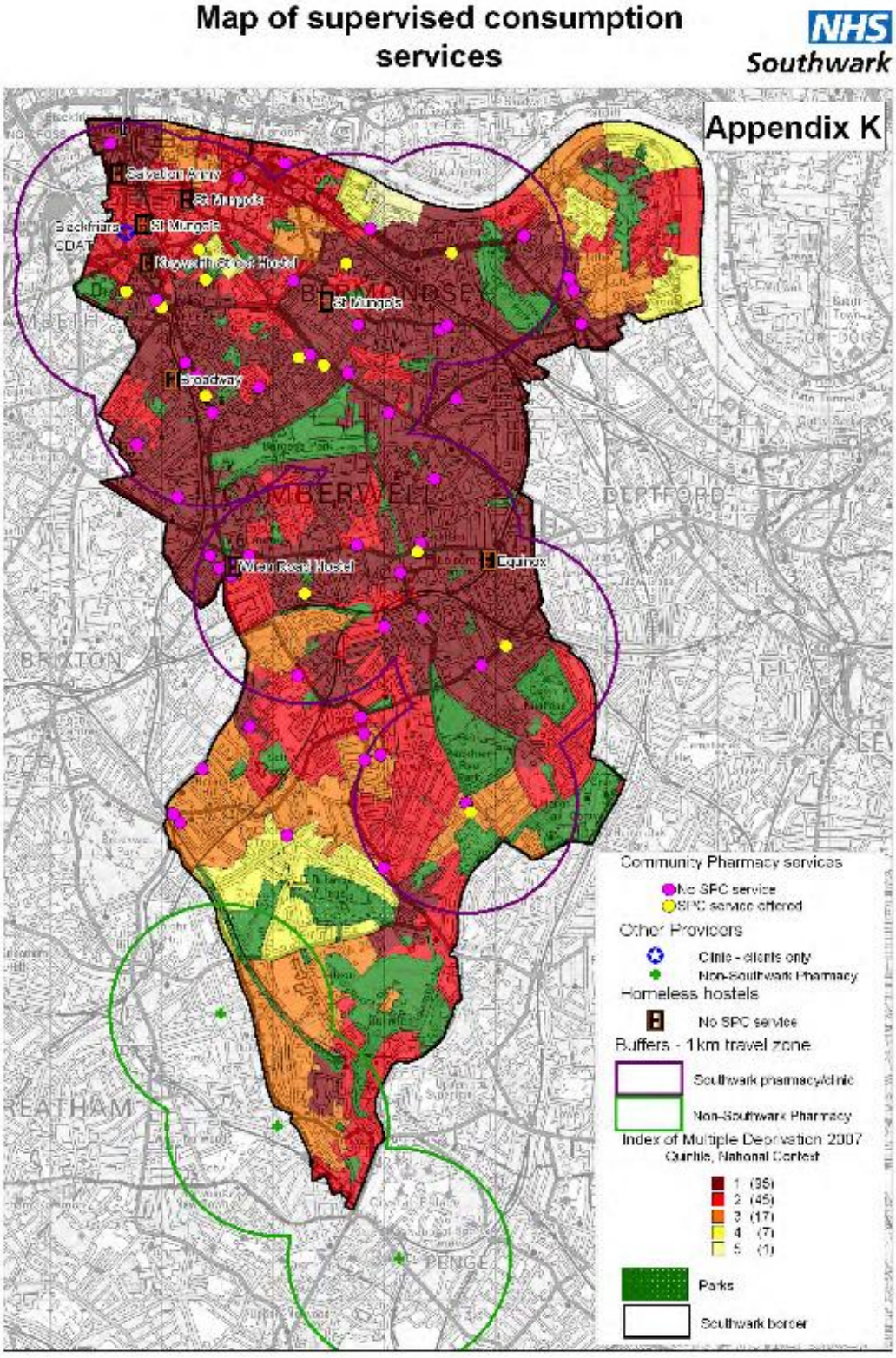
Southwark has a diverse population of approximately 288,200, of which the predominant ethnic group is White British (52.6%). According to the latest available figures, approximately 43% of Southwark's registered population falls within the ages of 25 to 44 years, compared to 28% for England as a whole.

According to the 2007 Index of Multiple Deprivation (IMD), Southwark is the ninth most deprived borough in London, although 45% of the population in Southwark is considered highly qualified for employment, which is well above the national average (29%). Approximately 14,000 people are of working age and are estimated to be unemployed in Southwark. Southwark also has 13% of residents who are considered to have no job qualifications, a little over the national average of 12%.

In 2009 it was estimated that 12,168 individuals aged 18 and over in Southwark were drinking at higher risk, while 6,199 individuals were dependent drinkers. From 2011 to 2012, 4,818 alcohol-related hospital admissions were reported in Southwark, which was an increase of 54% over the period from 2008 to 2009. Regarding substance misuse, NHS Southwark estimated in 2009 that more than 3,000 individuals in the borough used opiates and over two-

thirds of these individuals also used crack cocaine. Of these, more than 1,000 people were estimated to be using drugs by the intravenous route and therefore to be in need of access to needle exchange services. As a result, the borough of Southwark has set a priority on commissioning substance misuse services from a range of providers.

Figure 3-1 *Map with locations of supervised consumption of methadone services in Southwark (Southwark Council, 2011).*



At present, NHS Southwark commissions a supervised consumption service from community pharmacies and other providers in order to ensure compliance with an agreed action plan. The service includes dispensing opioid substitution medication in specified instalments and ensuring that each supervised dose is correctly consumed by the patient. Thirteen community pharmacies (21% of those in Southwark) provide a supervised opioid substitution service, providing either methadone or buprenorphine to clients as prescribed by GPs or substance misuse clinics. Figure 5-1 shows a map of Southwark with locations offering supervised consumption, including the Blackfriars Road Community Clinic, where the study was conducted.

3.2 Setting

The South London and Maudsley (SLAM) NHS Foundation Trust is the main provider of substance misuse and mental health treatment for South-East London. Recruitment was carried out by one of the Trust's Community Drug and Alcohol Teams (CDAT) at Blackfriars Road Clinic. CDAT was established in 1990 as part of the Lewisham and Guys Health Service. In 1999 the two health services were merged as part of the creation of SLAM; however, two community treatment facilities, Marina House and Blackfriars Road Clinic, which continued to operate from two separate sites until community drug services at Marina House were discontinued in 2010.

Blackfriars Road Clinic is currently part of the Southwark Treatment and Recovery Partnership (STARP). The Clinic provides a range of services, including assessment, advice, referrals, treatment interventions (including needle exchange) and aftercare for individuals suffering from substance misuse-related problems. The site also provides opioid substitution therapy. The service includes a daily duty service (1pm to 4:30pm), except on Tuesdays. Daily brief assessment is available between 9:30am and 12:15pm. Also, methadone

dispensing is available to some clients daily between 10am and 12pm, Monday through Friday.

3.3 Ethical approval

Ethical approval for the research was sought from the NHS Research Ethics Committee (Research Ethics Committee of London-Fulham (12/LO/0762)) (see Appendix A) to ensure that the participants and the researcher were protected and that the research was viable and ethical. All participants were provided with an information sheet that outlined the purpose of the study and the method of data collection (see Appendix B). The information sheet also explained that all data received were confidential and that participants can withdraw from the study at any time with no explanation required and no impact on their future treatment. The information sheet also outlined the incentive in the form of a £10 food voucher to be provided to participants after the initial interview, as well as £5 for each consecutive interview. The participants were then asked to sign a consent form stating that they understood the information sheet and that they agreed to take part in the study. An application for extending the end date of the study was granted to ensure further data collection for Study 1.b.

3.4 Research Plan

The research consisted of a series of three cross-sectional studies designed to examine the usefulness of a variety of monitoring tools in the assessment of methadone treatment compliance and/or outcomes, as follows:

- Study 1.a investigated the utility of urinary methadone to EDDP ratio in assessing compliance with daily methadone treatment. Study 1.b is a case series study investigated urinary methadone to EDDP ratio in assessing the compliance in daily

methadone treatment and change of the ratio during induction period.

- Study 2 investigated the utility of alcohol biomarkers Ethyl glucoronide (EtG) and Ethyl Sulphate (EtS) in screening for recent alcohol consumption in patients collecting their daily methadone doses.
- Study 3 investigated the drink driving cut-off limit used in connection with the alcohol breathalyser as a screening tool for patients presenting with problematic alcohol consumption.

3.5 Recruitment

Recruitment took place at the Blackfriars out-patient clinic. Potential participants were identified by key workers in the clinic and were subsequently approached by the researcher. This most often occurred when participants were at the clinic to receive their daily dose of methadone mixture or to collect their methadone mixture prescriptions. Participants would be undergoing supervised cmethadone onsumption and when they were collecting their methadone prescription participants were receiving their methadone dose supervised at the clinic. During a short initial meeting, the researcher briefed potential participants about the study. If they expressed an interest in taking part, the participants were escorted to a private room, where they were invited to read the information sheet and asked to provide written consent (see Appendix C). Both the information sheet and the consent form stated that the research was confidential and that there would be no further contact between the researcher and participants after the completion of the questionnaire.

Although there was a potential of interest if participants were presenting with withdrawal symptoms, the researcher accommodated further weekly sample collection by allowing the participant to come a day after or before the inteded day of collection.

A random sample of participants reported methadone dose was checked with their patient journey records to ensure that the dose matched and to avoid conflict in the results.

3.6 Statistical analysis

A power analysis was employed to establish the number of participants needed in each group to make the study statistically significant. Based on previous research, 31% of patients receiving methadone were understood to have problematic alcohol consumption (Senbanjo et al., 2007). Thus, study B (methadone and alcohol drug interaction) it was estimated that approximately one-third of the participants would present with dual dependence (Srivastava et al., 2008). Using the Raosoft online Sample Size Calculator, given a 5% margin of error and a confidence level of 95%, and with an estimated population size of 200, the minimum recommended sample size to achieve a meaningful result was estimated to be 132 patients.

1.6 Data analysis

Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS), Windows® version 15.0. Data were collected, coded and transferred to SPSS. The descriptive data, i.e., the demographic and background information, as well as the characteristics of licit substances and prescribed medication use, were analysed by calculating the frequencies, standard deviation, means and percentages of participant responses. Basic demographic and background information, including patients' age, sex, ethnic background and employment status, were compared using a chi-square test. A t-test was used to compare the continuous variables, including the severity of withdrawal symptoms at discharge and admission (see de Wet et al., 2004). When data were not normally distributed, a non-parametric test was utilised. A Mann-Whitney U test was used to compare distribution of continuous unpaired

variables, while a Wilcoxon signed rank test was used to compare the distribution of paired variables. Data that were missing were excluded from the analysis.

3.6.1 Inclusion criteria

Inclusion criteria differed slightly for each study, but all participants had to meet the following criteria:

- Diagnosed with heroin/opioid dependence
- Receiving methadone maintenance treatment for at least four weeks
- Between 18 and of 65 years of age.

3.6.2 Exclusion criteria

The following were excluded from all studies:

- Pregnant participants were not recruited due to different pharmacokinetics of methadone
- Participants unable to understand English
- Participants who were under the influence of substances to a debilitating degree that could affect their responses to the questionnaire or present a risk to the safety of the researcher, themselves or others
- Participants receiving opioid substitution treatment other than methadone
- Participants suffering from severe psychiatric problems.

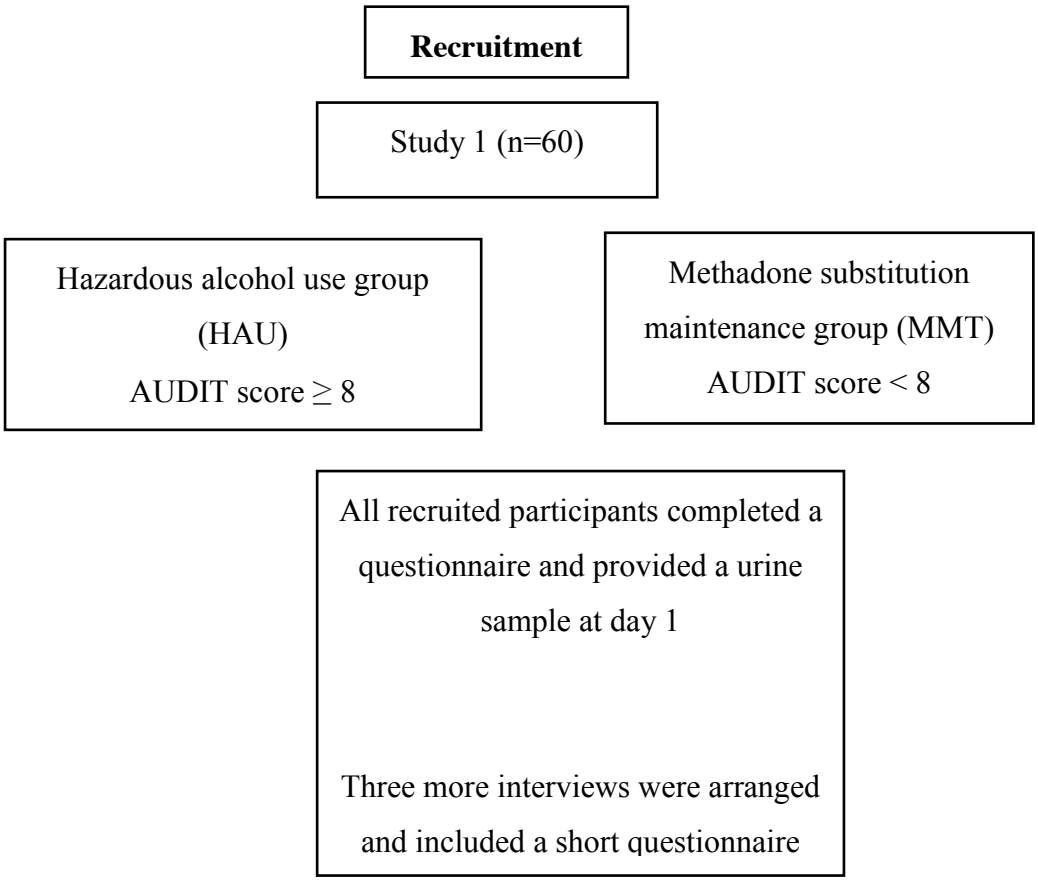
3.7 Study 1.a: Urinary methadone to EDDP ratio in patients receiving methadone substitution maintenance treatment

3.7.1 Design and Purpose

Using a cross-sectional prospective cohort design, Study 1 investigated the relationship between methadone and EDDP during fixed daily dosing in patients receiving methadone substitution maintenance treatment in order to assess whether the ratio of EDDP to methadone could be used effectively as a biomarker for methadone compliance. The setting, inclusion, and exclusion criteria have been described above unless stated otherwise (see sections 5.5 and 5.10).

3.7.2 Recruitment and Procedures

Flow chart of recruitment



Recruitment took place in the outpatient clinic, where the participants were identified by the clinical consultant or members of staff. The researcher approached potential participants (after consultation with the clinic team) and briefed them about the study. Participants in the study were first asked to complete a demographic and background information questionnaire (see Appendix D). This questionnaire included questions related to age, route of referral, ethnicity, and gender. The questionnaire also included questions about licit drug use, including cigarettes/tobacco and alcohol use. This was followed by a urine sample collection. The interview and the urine sample collection took 30 minutes in total. Depending on which study the participant belonged to, further appointments were arranged.

Participants were typically re-interviewed during a subsequent visit to the clinic to collect their methadone prescriptions or to receive their daily methadone dose. A shorter version of the questionnaire was administered, which included a self-report of methadone and alcohol use in the past 24 hours and the use of any illicit drugs. A SOWS questionnaire was also included, a urine sample was collected and appointments for subsequent interviews were set.

3.7.3 Research Tools

Each participant was asked, with the assistance of the researcher, to complete a questionnaire that covered general demographic information (Appendix D), including questions related to age, route of referral, ethnicity and gender. The questionnaire also included questions about licit drug use, including cigarettes/tobacco and alcohol use, as well as questions regarding methadone intake and dosage.

Participants were also asked to complete the following validated research tools:

The Alcohol Use Disorders Identification Test (AUDIT)

The Alcohol Use Disorders Identification Test (AUDIT) has been widely used as a screening tool to determine level of alcohol consumption. Developed by The World Health Organization (WHO), the AUDIT is a self-report instrument that is used to identify harmful and hazardous alcohol use in the past year (Saunders et al., 1993). Although the AUDIT was developed for use with primary care patients, the instrument's use has been expanded to include the prediction of alcohol withdrawal symptoms (Dolman et al., 2005). All of the items address questions related to the preceding year, with the exception of the first two. The first three questions assess alcohol intake. Questions 4 to 6 enquire about aspects of alcohol dependence. Questions 7 and 8 address adverse reactions to drinking, and the final questions address alcohol-related problems.

The items are scored on a scale from 0 to 4, on which participants report the frequency of various actions or effects, resulting in a total possible score of 40 (see Allen et al., 1997). The AUDIT total score helps healthcare professionals determine whether an individual's alcohol consumption is at hazardous level (8-15), harmful level (16-19), or alcohol dependent (20 or more). Studies have confirmed the validity and sensitivity of the AUDIT test (Allen et al., 1995).

A modified shorter version of the AUDIT, which consists of the first three questions, is known as the AUDIT-C. Each item is scored on a scale from 0 to 4, resulting in a total possible score of 12. In men, a score of 4 or more is considered positive for identifying hazardous drinking or alcohol use disorders, while a score of 3 or more in women is considered positive for identifying hazardous drinking (Bush et al., 1998).

Short Opiate Withdrawal Symptoms Scale (SOWS)

Participants also completed the Short Opiate Withdrawal Symptoms Scale (SOWS), a ten-item version of the Opiate Withdrawal Scale (OWS). SOWS has been validated as an

effective tool for assessing opiate withdrawal symptoms in clinical settings, as well as for research purposes, and it has been used during detoxification (Bearn et al., 1996; de Wet et al., 2004; Gossop et al., 1990). The ten symptoms evaluated by SOWS include feeling sick, stomach cramps, muscle spasm or twitching, feeling of coldness, heart pounding, muscle tension, aches and pains, yawning, runny eyes, and insomnia and other sleeping problems.

The scoring of the tool is based on a numerical Likert rating scale, with 0, 1, 2, and 3 used to designate, respectively, *none*, *mild*, *moderate*, and *severe* symptomology. Hence, the maximum score obtainable, indicating the highest level of severity of withdrawal symptoms, is 30.

The Treatment Outcome Profile (TOP)

The Treatment Outcomes Profile (TOP) was developed by the National Treatment Agency for Substance Misuse (NTA). It is a brief instrument that consists of twenty reliable, valid scale and combined-item period prevalence measures. It records the frequency of illict drug use 28 days before treatment. The TOP records four main areas affecting the opioid dependent's life:

1 *Substance use*: the participant is asked about the number of days of use of alcohol, illicit opiates, crack cocaine, cocaine powder, amphetamines, cannabis and any other substance use in the past 28 days. During the development of TOP, estimates of the total quantity used on a typical day were recorded as follows: alcohol in standard drinks (1 UK unit = 10 ml ethanol by volume); verbatim reports of quantity used or money spent, based on estimated averaged street prices for heroin (£10 = 0.2 g); crack cocaine (£10 = 0.1 g), powder cocaine (£50 = 1.0 g), amphetamine sulphate (£10 = 1 g), cannabis (number of 'joints' and/or pipes) and benzodiazepines (converted to 5-mg diazepam equivalents). The participant was also asked

about the number of injecting days in the past 28 days and about the prevalence of needle/syringe sharing.

2 *Health*: Includes a participantive rating score between 0-20 of physical and psychological health status.

3 *Crime*: participants are asked about the number of days they have committed shoplifting, other theft or drug selling, as well as about the prevalence of vehicle, property and fraud and assault/violent crime.

4 *Social functioning*: participants are asked to rate their quality of life on a scale between 0-20. They are asked about the number of days of paid work and attendance at education/training and about housing problems or risk of eviction (Marsden et al., 2008). The TOP also measures change and progress in these key areas at different stages of treatment in order to assess treatment effectiveness and progress made; outcomes at different stages can be measured by completing the TOP at a specific stage of the treatment. At the start of treatment, the TOP can provide important information about clients' drug use before treatment and can act as a baseline for comparison with subsequent TOPs. During the treatment, the TOP can be used as part of good practice for regular care plan reviews that are usually completed in 12 week (3 month) cycles.

NDTMS requires a TOP to be reported every 26 weeks (6 months). At the end of the treatment, it is recommended that a TOP be completed within two weeks either side of a client leaving structured treatment. The TOP form may also be completed after a client leaves treatment, which will assist providers to measure the longer-term impact of their treatment (Marsden et al., 2010).

In this study, the TOP was used during treatment to assess methadone treatment compliance by collecting information related to the patient's illicit drug use in the past month, including injecting behaviour. Other parameters reported, including crime, psychological wellbeing and housing issues, were also documented.

3.8 Study 1.b: Methadone:EDDP Ratio During Induction

3.8.1 Design and Purpose

Using a case series study, the ratio of methadone:EDDP was measured during fixed daily dosing in patients who began receiving methadone substitution maintenance treatment (induction period) in order to assess whether the ratio of EDDP:methadone could be used effectively as a biomarker for methadone compliance and whether the ratio changes during the induction period. The setting has been described above unless stated otherwise (see sections 5.5).

3.8.1.1 Inclusion criteria

Participants had to meet the following criteria:

- Diagnosed with heroin/opioid dependence
- Started receiving methadone maintenance treatment in the past 24 hours
- Between 18 and of 65 years of age.

3.8.1.2 Exclusion criteria

The following were excluded from this study:

- Pregnant participants were not recruited due to different pharmacokinetics of methadone
- Participants unable to understand English

- Participants who were under the influence of substances to a debilitating degree that could affect their responses to the questionnaire or present a risk to the safety of the researcher, themselves or others
- Participants receiving opioid substitution treatment other than methadone
- Participants suffering from severe psychiatric problems
- Participants who have been receiving methadone for more than 24 hours.

3.8.2 Recruitment and Procedures

Recruitment took place in the outpatient clinic, where the clinical consultant or members of staff identified the participants who just started receiving their daily methadone on the previous day. The researcher approached potential participants (after consultation with the clinic team) and briefed them about the study. Participants in the study were first asked to complete a demographic and background information questionnaire (see Appendix D). This questionnaire included questions related to age, route of referral, ethnicity, and gender. The questionnaire also included questions about licit drug use, including cigarettes/tobacco, and alcohol use. This was followed by a urine sample collection. The interview and the urine sample collection took 30 minutes in total. The participants were asked to meet the researcher when they visit the clinic for their daily methadone dose for the next 13 days. Participants were re-interviewed during the subsequent visits to the clinic and a shorter version of the questionnaire was administered (see study 1.a).

The research tools are the same as the ones used in study 1.a (see Appendix D).

3.8.3 Biological samples

A urine sample was collected after the interview. All samples were collected at trough, i.e., before administration of the daily dose of methadone, in 20 mL universal tubes. One sample was collected weekly from each participant for four weeks. Participants were provided with a urine bottle and were asked to void unobserved. The urine samples were frozen in the laboratory at -20° C until analysis. At the end of the interview, each participant was assigned an appointment for his or her next interview. The whole procedure, including the structured interview, took 30-40 minutes.

Quantifying methadone concentrations in biological fluids depends upon the application of specific, sensitive and rapid analytical methods (Fernandez et al., 2010). Various separation methods have been developed, such as gas chromatography (GC), liquid chromatography (LC) and high performance liquid chromatography (HPLC), which can be combined with such detection methods as ultraviolet light (UV) and mass spectrometry (MS) (Shakleya, 2007). Capillary electrophoresis is a recently developed method that has not yet been used extensively.

1) Gas Chromatography (GC)

Coupled with other methods of detection, including nitrogenous phosphorous, electron capture and mass spectrometry (GC/MS) using positive chemical ionization (PCI), has proven successful in the analysis of methadone and its metabolite (EDDP) in serum, plasma or whole blood (Gunnar et al., 2006). However, the application of GC requires an extensive derivatisation of the methadone sample, which can be considered a drawback. Gunnar et al. have developed methodologies to make GC-based techniques with MS faster and more cost-efficient in determining levels of methadone and its metabolite in the blood, and their results indicate a good linearity and accuracy with an LOQ of 25 ng/ml. Earlier, Bermejo et al.

(1998) used GC/MS to determine methadone levels in urine and plasma from patients under detoxification treatment and achieved mean recoveries of 64.30% from urine and 79.03% from plasma. However, recent studies have used LC as the method of choice in determining methadone in plasma for various purposes, including in TDM.

2) High performance liquid chromatography (HPLC)

Compared to GC, the HPLC analytical method requires less sample preparation, which makes this method more practical and appealing. Like GC, HPLC methods, coupled with MS and UV detectors, have been successfully applied in measuring methadone in the blood, serum and plasma (Rook et al., 2005). However, HPLC/MS is considered the method of choice for TDM, especially when monitoring MMT patients, because of the possibility of co-medication and the consumption of illegal drugs in this population (Baumann et al., 2004; Fernandez et al., 2010).

Table 3-1 *Summary of Studies that Conducted Methadone Analysis using LC Analytical Methods and Applying Various Chromatography Conditions*

Author	Matrix	Method	Sample preparation	Column	Other chromatography conditions
Rook et al., 2005	Plasma	LC–MS/MS	Solid phase extraction using mixed mode sorbent columns (MCX Oasis)	Reversed phase Zorbax column with a gradient	Mobile phase: consisting of ammonium formate (pH 4.0) and acetonitrile. The run time was 15 min. The method was validated over a concentration range of 5–500 ng/mL for all analytes.
Fernández et al., 2005.	Plasma	HPLC–DAD		250 mm × 4.6 mm i.d. XTerraRP8 column of 5 μm particle size	Mobile phase: was acetonitrile −0.02 M phosphate buffer pH 6.53 and eluted in the gradient mode.
Choo et al., 2005.	Human meconium	LC–APCI–MS/MS	Solid-phase extraction using a modification of the ElSohly method.	Synergi Hydro-RP 80A (50 mm × 2.0 mm, 4 μm), fitted with a C18 ODS Octadecyle (4.0 mm × 2.0 mm) guard column.	Mobile phase:(gradient elution), consisting of (A) 10 mM ammonium formate in water with 0.001% formic acid (pH 4.5) and (B) acetonitrile, at a flow rate of 300 μL/min.
Quintela et al., 2006.	Plasma	LC/MS	Automated solid-phase extraction using Gilson Aspect XL	3.5 μm (30 mm × 2.1 mm I.D.) reversed-phase column	Mobile phase: was a gradient of acetonitrile in 0.1% formic acid programmed as follows: 18% acetonitrile during 0.5 min, increased to 60% in 2 min and decreased to 18%.
Shakleya et al., 2007.	Plasma	LC–APCI-MS/MS		Synergi Hydro-RP 80A (50 mm × 2.0 mm, 4 μm) column with an identically packed guard column (4 mm × 2.0 mm)	mobile PHASE: Gradient elution with (A) 10 mM ammonium acetate in water, 0.001% formic acid (pH 4.5) and (B) acetonitrile at a flow rate of 200 μL/min was used with a gradient program of 40% B for 2 min, increasing to 90% over 7 min and hold for 2 min. The HPLC column was re-equilibrated for 6 min, giving a total run of 17 min.
Goucher et al., 2010.	Urine, plasma and fingerprints	LC-MS/MS	Solid Phase extraction using Waters Oasis MCX 60 mg cartridge.	Tosoh TSK-Gel Amide-80 3 μm (250×4.1 mm) HILIC carbamoyl phase column	The mobile phase: isocratic mobile phase consisted of 28:72 v/v acetonitrile + 0.01% formic acid/3 mM ammonium formate in water + 0.01% formic acid.

3.8.4 Analysis of urinary methadone and EDDP

Urine samples were transported by the researcher from the clinic in a courier bag to the laboratory at the Institute of Pharmaceutical Science at King's College London and were stored at -20^{o} C. Urine samples were analysed using High Performance Liquid Chromatography and Ultraviolet Detector (HPLC/UV) to measure methadone and EDDP concentrations.

3.8.4.1 HPLC/UV determination of urinary methadone and EDDP

The method originally developed by Wolff et al. (1997) for analysing methadone and EDDP in urine was optimized using HPLC/VU based on the methodology for liquid-liquid extraction (LLE) and HPLC-UV. The method has been slightly modified.

3.8.4.2 Materials and Methods

Reagents

Methanol, acetonitrile, formic acid and propan-2-ol were purchased from Fisher Scientific (Loughborough, UK). Ammonium formate, 1-chlorobutane, ammonium hydroxide, 1,2-dichloroethane and ammonium perchlorate were obtained from Sigma-Aldrich (Steinheim, Germany). Sodium hydrogen carbonate, sodium carbonate and ammonium acetate were obtained from BDH Laboratory Supplies (Poole, UK). Ultra-pure water (UPW) (18.2 MΩ·cm) was obtained from a Millipore Milli-Q water purification system (Bedford, MA, USA). (±)-Methadone hydrochloride (MTD), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine perchlorate (EDDP) and Benzhexol (BZX) internal standard were purchased from Sigma-Aldrich.

Specimen collection

Pre-dose urine samples (20 ml) were collected from clients receiving methadone substitution maintenance treatment. The samples were transported by the researcher from the clinic in a courier bag to the laboratory at the Institute of Pharmaceutical Science at King's College London and were stored at -20^{o} C.

Analytical methods

Stock solutions of MTD, EDDP and internal standard BZX were prepared at 1 mg/mL in methanol and stored at 4° C. Working solutions were prepared each day containing each component by appropriate dilution, with UPW, methanol or urine, and pH adjustments made depending on the analysis. Mixed standards were prepared at 10 to 500 ng/mL (n=11) in urine and UPW for calibration studies. Replicate samples at 50 ng/mL (n=6) were prepared at pH 7, 11 and 3 by adjustment with ammonium formate or formic acid, to determine recovery. Standard solutions of analytes at varying concentrations were prepared in UPW or methanol for chromatographic peak identification.

Liquid-liquid Extraction

A buffer solution was prepared for Liquid-Liquid Extraction (LLE). The buffer at pH 10 was prepared by mixing 1 mol/L sodium carbonate in UPW with 1 mol/L sodium hydrogen carbonate (7:3), and the ratio adjusted, if necessary, to yield a solution of pH 10. Water saturated 1-chlorobutane was prepared by addition of 100 mL 1-chlorobutane to a small amount of water in a separating funnel and shaken well until saturation was achieved.

The extraction procedure was performed by adding 0.5 mL sodium carbonate buffer

to 15 mL glass conical tubes with glass stoppers. Then, 2 mL samples were aliquoted to the buffer before vortex mixing for 20 seconds. In a fume hood, 5 mL of water-saturated 1-chlorobutane was added. The tubes were stoppered, transferred to a tube shaker and mixed mechanically for 15 minutes. Samples were then centrifuged at 4° C for 10 min. at 2,100 rpm. The chlorobutane upper layer was transferred to glass tubes and evaporated to dryness under clean air. A further 5 mL of water saturated 1-chlorobutane was added to the lower layer and participanted again to the above mechanical mixing and centrifugation steps. The second upper layer was then transferred to the residue from the first extraction and evaporated again. Extracts were then reconstituted in 100 µL methanol, transferred to 300 µL conical vials, capped and stored at 4° C until analysis.

Normal phase LC (NPLC) was performed on a Gilson 234/307 auto-sampler with 151 UV/VIS detector (Bedfordshire, UK) fitted with an Apex-I silica column (5 µm particulate silica 250 x 46 mm) (Mid Glamorgan, UK). Extracts (50 µL) were injected in an isocratic mobile phase recycling at 2 mL/min. consisting of methanol, 1,2-dichloroethane, propan-2-ol and 40 % v/v ammonium perchlorate in UPW (90.5:5:4:0.5). The mobile phase was filtered and degassed and re-made every week. Diode array detection was set to 215 nm.

Method performance

For validation, the method was evaluated in terms of linearity, accuracy and repeatability, limit of detection (LOD) and limit of quantification (LOQ), as specified by the U.S. Department of Health and Human Services. Linearity was determined by preparation of calibration standards in UPW and in urine for matrix matched analysis. Standard addition was employed and concentrations of 10-500 ng/mL (n=6) of MTD

and EDDP were spiked into UPW and urine before extraction by LLE and LC-UV analysis. A calibration curve was produced from the chromatogram peak areas produced by the analytes at different concentrations. The regression line was calculated using the least squares method and expressed by the coefficient of correlation (R^2), with values of over 0.98 taken to be acceptable. In order to determine extraction recovery, UPW and urine samples (n=6) were spiked to 200 ng/mL concentrations participanted to extraction procedure and compared to matrix-matched standards. Recovery was calculated by comparison of the peak areas before and after extraction and expressed as a percentage.

Precision was evaluated as the coefficient of variation (% CV) measured between recoveries of spiked urine extracts (n=6) and accuracy by percentage comparison of the ratio of achieved concentration to nominal concentration in a standard. Sensitivity was determined as LOD and LOQ. The LOD is the lowest concentration of analyte that can be detected qualitatively by the procedure. The LOQ is the lowest amount that can be quantitatively determined, and hence quantitation below this value is not deemed reliable. Both limits are determined by calculating the signal to noise (S/N) ratio from the height of the chromatographic peak compared to the highest and lowest peaks of baseline noise and applying the 3:1 S/N for LOD and 10:1 for LOQ.

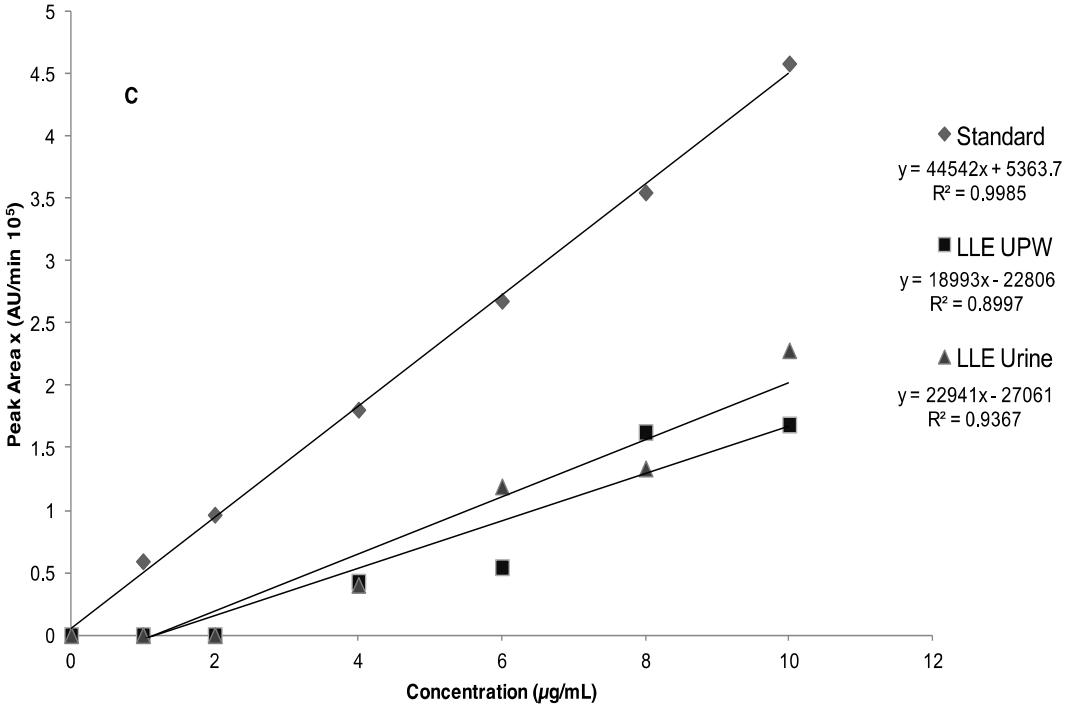


Figure 3-2 *Calibration plot for MTD following LLE from ultra-pure water or urine*

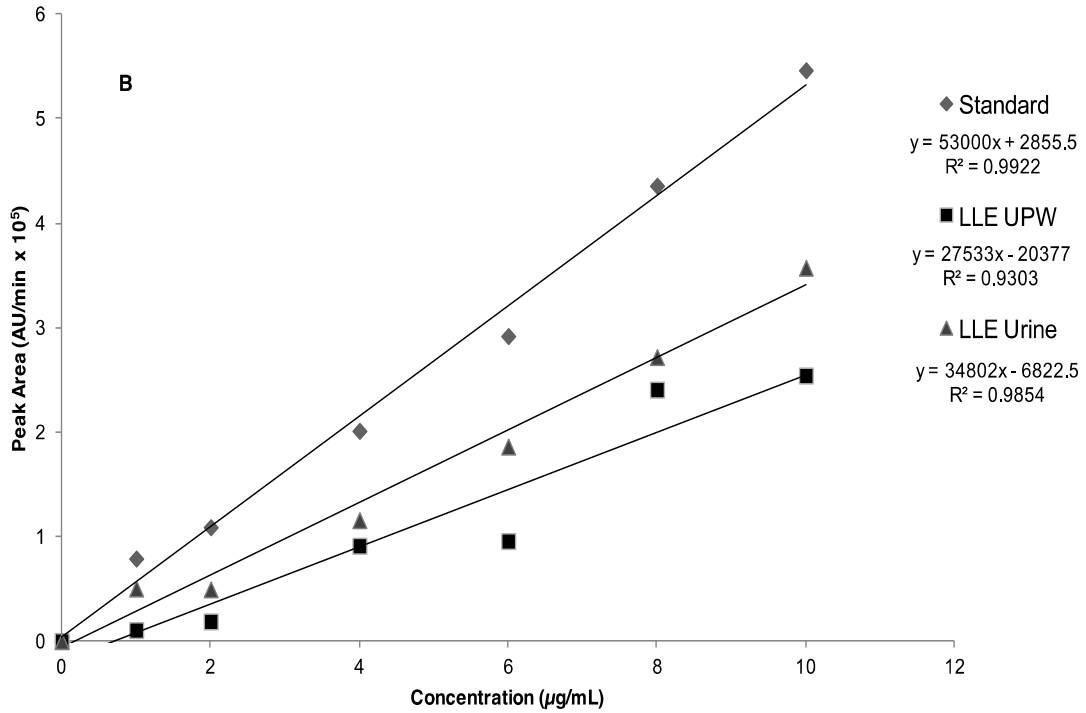


Figure 3-3 *Calibration plot to show EDDP response following LLE extraction from ultra-pure water or urine*

3.9 Study 2: Recent Alcohol Consumption Biomarkers (EtG and EtS) in Patients Receiving Methadone Substitution Maintenance Treatment

3.9.1 Study Design and Purpose

Using a cross-sectional prospective cohort design, the study investigated the effectiveness of using the alcohol biomarkers Ethyl glucoronide (EtG) and Ethyl Sulphate (EtS) to screen for recent alcohol consumption in patients collecting their daily methadone dose. The purpose was to help establish whether these biomarkers can be used to indicate recent alcohol use among clients receiving methadone substitution maintenance treatment. The study setting, inclusion, and exclusion criteria have been described in sections 5.5 and 5.10, and recruitment has been described on page 106.

3.9.2 Research Tools

Each participant was asked, with the assistance of the researcher, to complete a questionnaire that covered general demographic information (Appendix D), including questions related to age, route of referral, ethnicity and gender. The questionnaire also included questions about licit drug use, including cigarettes/tobacco and alcohol, as well as questions regarding methadone intake and dosage. Alcohol-related questions in the self-report included the consumption of alcohol and illicit drugs in the past month using TOP and the consumption of alcohol, kind and amount in the past 24 hours.

3.9.3 Biological samples

A urine sample was collected using the same procedure as previously described and used to determine EtG and EtS levels. The analysis was carried out by the King's College Hospital Department of Clinical Chemistry using liquid chromatography coupled with tandem mass spectrometry (LC/MS-MS). A method adapted from that used by Helander & Beck (2005) was used to quantify EtG and EtS in standards and urine samples as described briefly below.

3.9.4 Analysis of urinary EtG and EtS

3.9.4.1 Materials and Methods

Reagents

All chemicals and solvents were of analytical grade. Ethyl-β-D-glucuronide was purchased from LGC Standards GmbH (Wesel, Germany). Ethyl sulphate sodium salt was purchased from Aurora Analytics (Baltimore, U.S.A.). Internal standards (d5-EtG and d5-EtS) were also purchased from Aurora Analytics. Acetonitrile was obtained from Rathburn Chemicals Ltd. (Walkerburn, Scotland). Formic acid and ethyl phosphate were purchased from Sigma-Aldrich Co (Poole, UK).

Equipment

A Jasco^{TM} LC 2000 HPLC system with three PU-2085 pumps, a MX-2080-32 solvent mixing module, an AS-1550 autosampler and a CO-2067 column oven (Tokyo, Japan) was used. This was coupled with a triple quadrupole mass spectrometer API 3200^{TM} (Applied Biosystems, Cheshire, UK), which could be operated with either an electrospray ionisation (ESI) or atmospheric pressure chemical ionisation (APCI) source.

Preparation of standards

Stock solutions of the standards were prepared as follows: d5-EtG 100 mg/L was prepared by weighing 1 mg of d5-EtG using a 5-point balance and adding it to a 10 mL volumetric flask, making it up with water. It was then stored at -10º C. d5-EtS 100 mg/L was prepared by weighing 1 mg of d5-EtG using a 5-point balance and adding it to a 10 mL volumetric flask and making up with water.

Calibration standards containing 0.05, 0.1, 1, 5, 10, and 50 mg/L of EtG + EtS were prepared by serial dilution in blank urine. A stock solution containing approximately 5 mg/L of internal standard (d5-EtG + d5-EtS) was prepared in water and stored at 4° C.

Sample preparation

Urine was mixed with 1:10 (v/v) with (EtG-d5 and EtS-d5). 100 µL of urine was mixed with 900 µL of internal standard solution and vortexed for 30 seconds, as described by Helander & Beck (2005). Samples were centrifuged at 10,000 RPM for 3 min. 900 µL of the supernatant was transferred to an HPLC vial.

3.9.5 LC-MSMS

EtG and EtS were chromatographically resolved using a Hypercarb column (100 x 2.1mm, 5µm; Thermo Scientific, Hertfordshire, UK). The mobile phase consisted of 25mmol/L formic acid with 5% acetonitrile and was pumped isocratically at 600µl/minute. This method was adapted from Stephanson et al. (2002) and was optimised and extended to allow detection of EtS as well as EtG. Analysis time per sample was 10 minutes. Injection volume was 10µl. Negative-ion mass spectra of the

eluates were recorded in MRM mode. Data were acquired and quantified using Analyst™ software version 1.4.2 (Applied Biosystems) and using peak area analysis corrected by comparison to the internal standard.

Liquid chromatography linearity

Linearity checks were carried out with aliquots of pooled blank urine spiked with a combined EtG/EtS stock at different concentrations and then serially diluted with pooled urine. A linear relationship was observed between 0.2 mg/L and 100 mg/L for EtG (see Figure 5-4) and between 0.1 mg/L and 80 mg/L for EtS (Figure 5-5), with good correlation. For EtS, linearity and precision were acceptable down to 0.05 mg/L. However, the signal to noise ratio at this concentration was unacceptable and therefore, for robustness, the LLOQ for EtS was set at 0.1 mg/L.

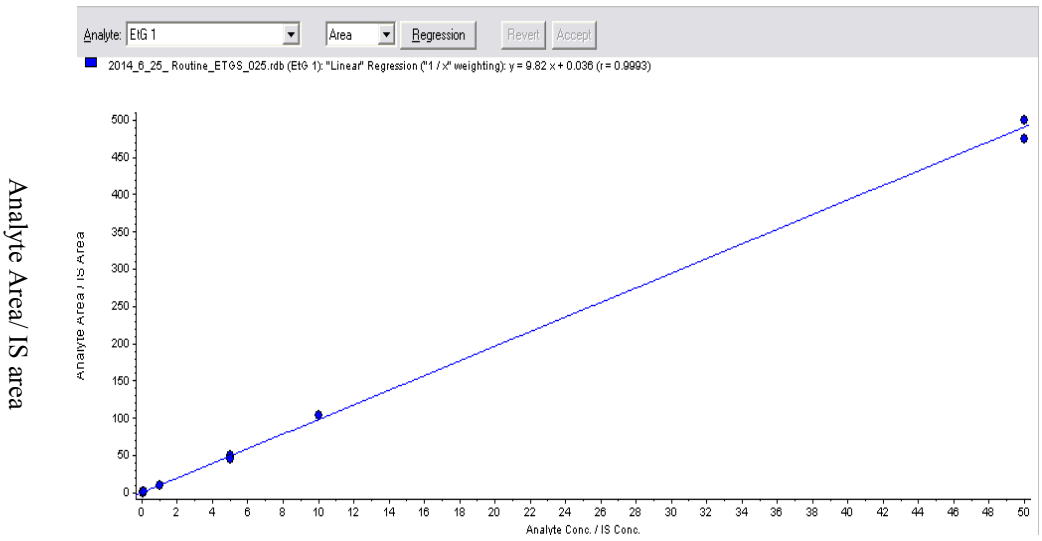


Figure 3-4 *EtG calibration curve – 6 standards – concentrations 0.05, 0.1, 1, 5, 10 and 50 mg/L. Standards run at the beginning and end of the batch*

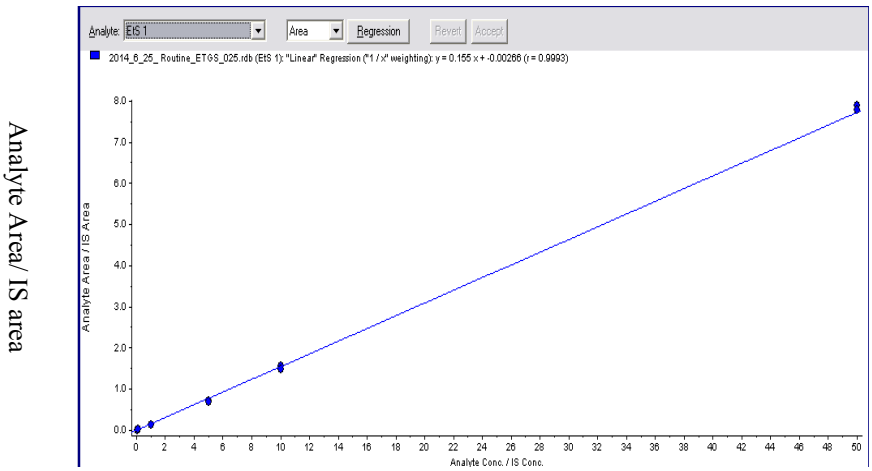


Figure 3-5 *EtS calibration curve – 6 standards – concentrations 0.05, 0.1, 1, 5, 10 and 50 mg/L. Standards run at the beginning and end of the batch*

The lower limit of quantification (LLOQ) can be defined as 'the point where there is a signal to noise ratio of at least 5:1 in addition to where 20 replicates generate precision within 20% and with accuracy of 80-120%' (U.S. FDA, 2001).

Recovery

To determine the recovery of the method, six urine samples were spiked with a mix of defined amounts of EtG/EtS standards prior to a ten-fold dilution in internal standard solution for analysis.

Figure 3-6 *Standard 3 (1 mg/L of each analyte)*

- Total ion count – each SRM in method a different colour (see next slide for further explanation

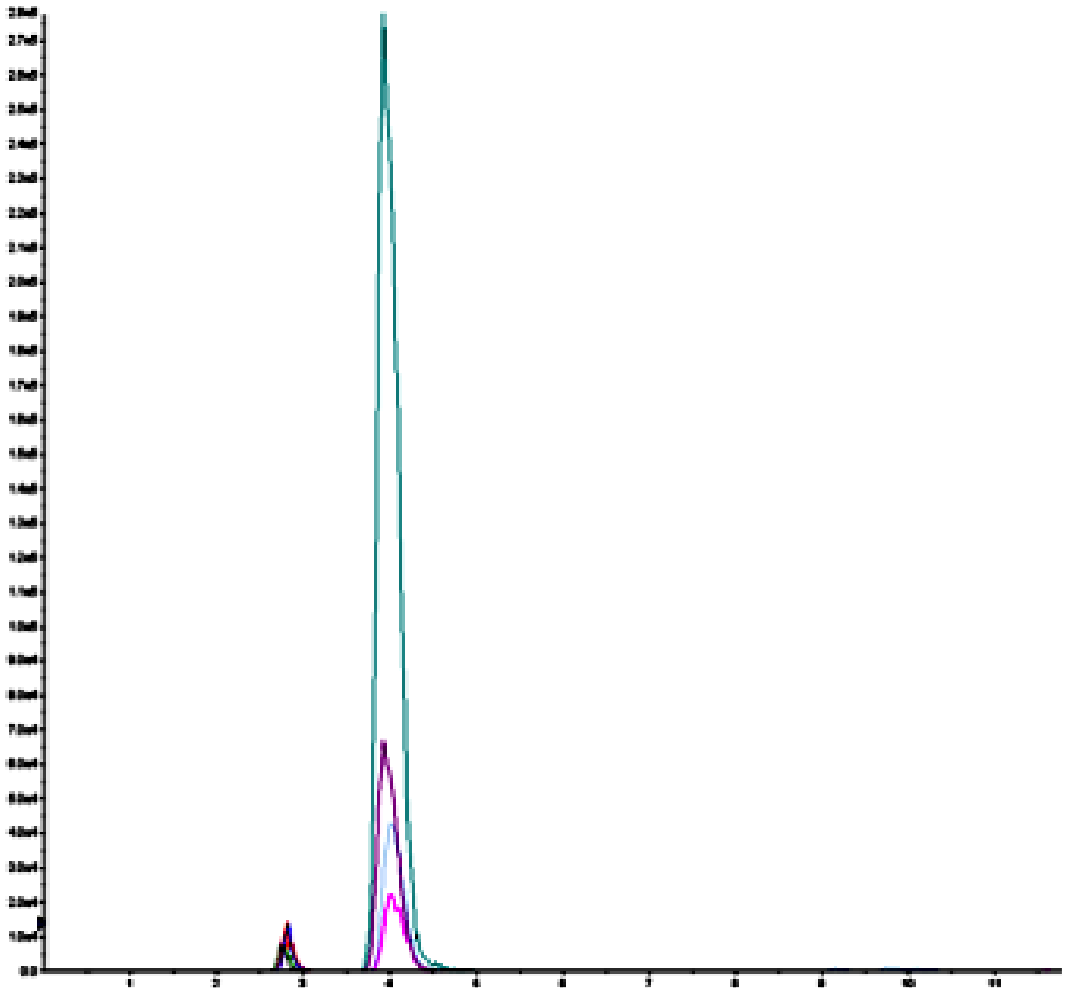


Figure 3-7 *Figure showing all SRMs monitored (top panel, each mass transition a different colour), then in bottom 4 panels, individual mass transitions for analyte (EtG or EtS) and their corresponding deuterated internal standard (EtG-d5 or EtS-d5) shown. Individual parent and daughter ions (Q1/Q3 masses) indicated on the plots.*

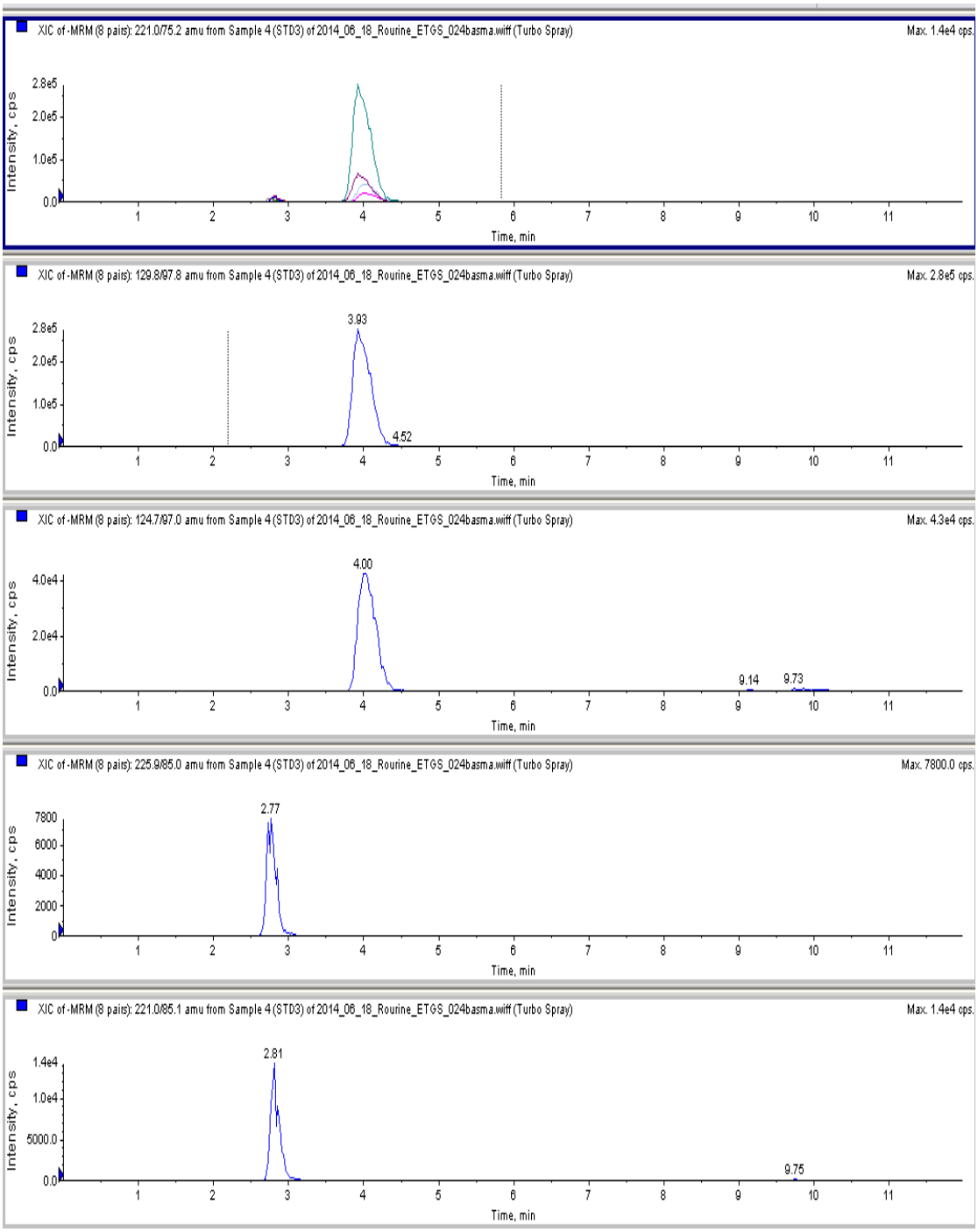


Figure 3-8 *Positive patient urine (EtG 13.1 mg/L and EtS 9.6 mg/L)*

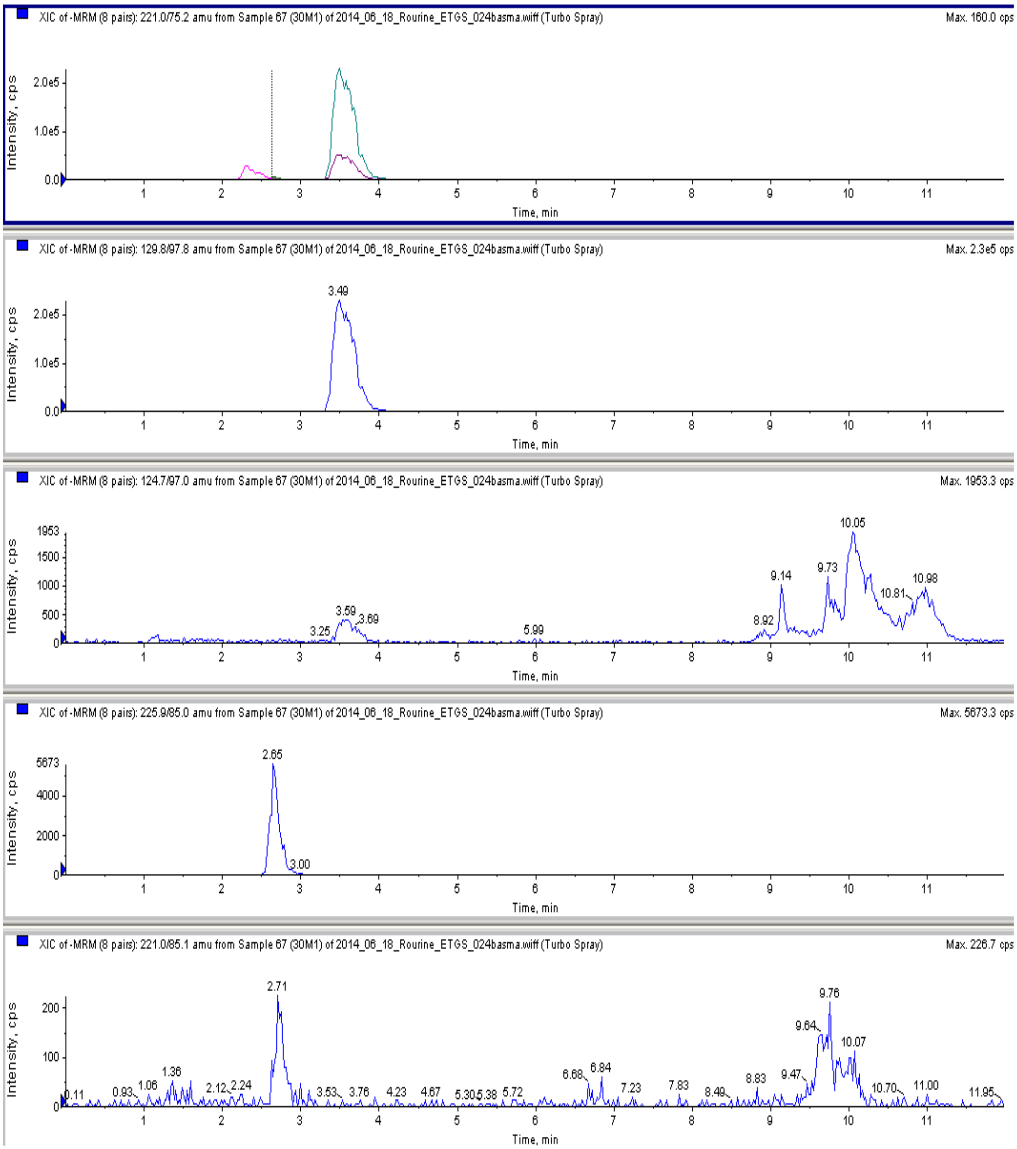
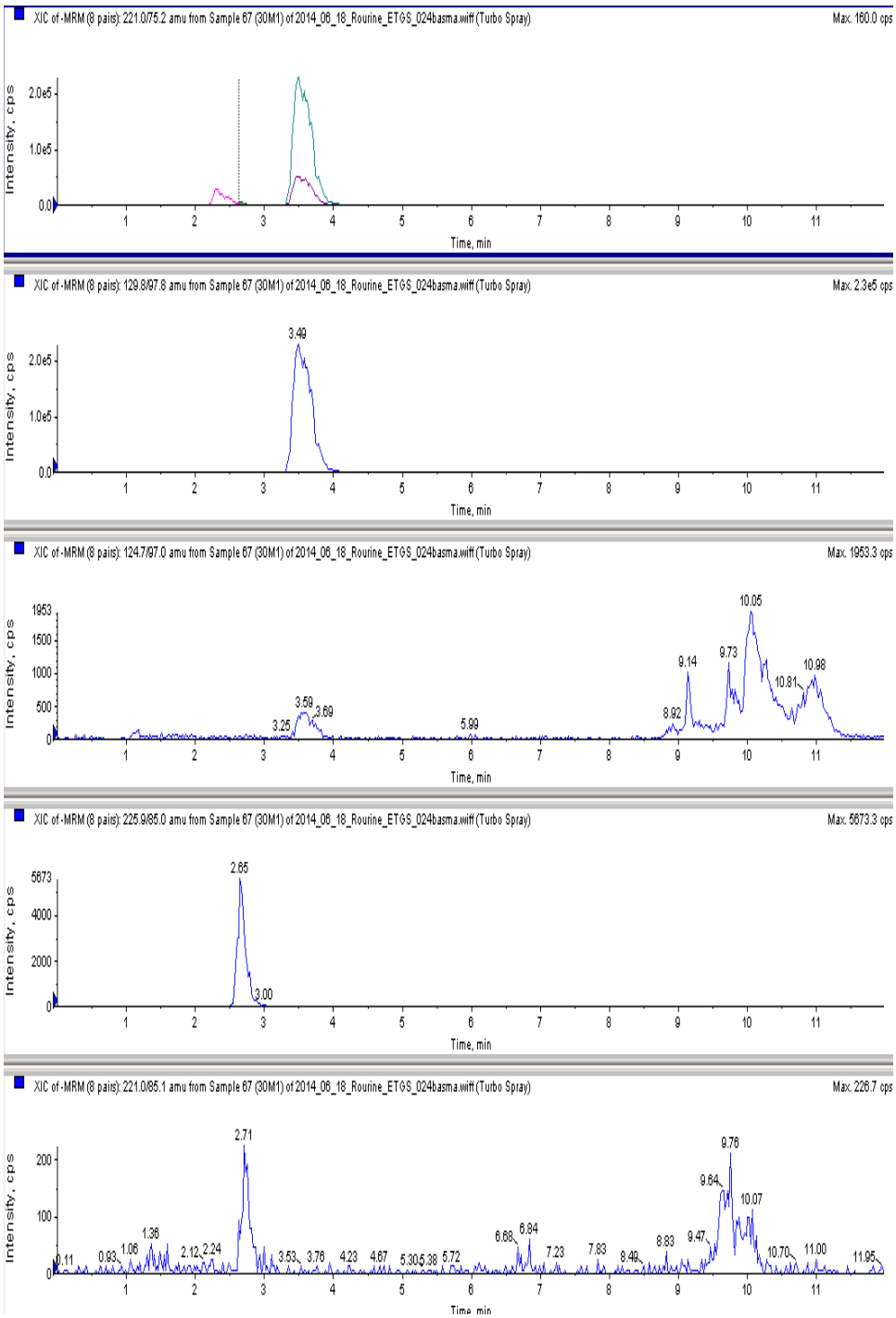


Figure 3-9 *Negative patient urine (EtG and EtS both <0.05 mg/L)*



3.10 Study 3: An investigation of the Use of the Breathalyser in Methadone Substitution Maintenance Treatment

3.10.1 Design and Purpose

Using a cross-sectional design, the study investigated breathalyser readings during fixed daily dosing in patients receiving methadone substitution maintenance treatment and presenting with problematic alcohol use. The goal was to help establish whether the current breathalyser cut-off limit for alcohol use is a suitable indicator for alcohol consumption among clients receiving methadone maintenance.

3.10.2 Setting

The setting was described above (see section 5.5). However, participants were alcohol users who were also prescribed methadone and participants were required to meet the following criteria in addition to those described in section 5.10:

- Participants present with problematic alcohol consumption.
- Participants who were breathalysed.

3.10.3 Recruitment

Recruitment took place in the outpatient clinic. The participants were identified by staff and were subsequently approached by the researcher during their visits to the clinic and briefed about the study. Upon expressing interest, potential participants were escorted to a private room and asked to provide written consent (see Appendix C).

3.10.4 Research Tools

Participants were asked to complete a research interview involving a questionnaire that included sociodemographic questions and questions pertaining to licit and illicit drug use, methadone treatment and other current treatment. The questionnaire also included questions related to alcohol use, including most recent alcohol consumption and type of drink. The interview also made use of AUDIT, SAWS and SOWS measures (Appendix F), including questions related to age, route of referral, ethnicity and gender. The questionnaire also included questions about licit drug use, including cigarettes/tobacco and alcohol, as well as questions regarding methadone intake and dosage. Alcohol-related questions in the self-report included the consumption of alcohol in the past month and the consumption of alcohol, kind and amount in the past 24 hours. Further sociodemographics and laboratory results were collected using patients' journey files.

Short Alcohol Withdrawal Symptoms Scale (SAWS)

The Short Alcohol Withdrawal Symptoms Scale (SAWS) is a ten-item psychometric scale that is relatively easy to administer. Five items on the SAWS assess a psychological component of withdrawal symptoms: anxiety, confusion, restlessness, misery and memory problems. The other five items concern physical components of withdrawal symptoms: tremors, nausea, heart pounding, sleep disturbance and sweating. The SAWS can be used to determine the severity of alcohol withdrawal symptoms at the first clinical assessment and can be administered for continuous assessment. In administering the SAWS, a numerical Likert rating scale is presented, with 0, 1, 2, and 3 used to respectively designate *none, mild, moderate*, and *severe* symptomology. A score of 30 was the maximum obtainable, and this score was later used in calculating the mean total withdrawal scores. This scale has been used in an

in-patient setting to assess the level of management and treatment effectiveness (Gossop et al., 2002).

Leeds Dependence Questionnaire (LDQ)

The Leeds Dependence Questionnaire (LDQ) is tool used to indicate how severely dependent a person is and to assess how difficult it will be to achieve a positive outcome. LDQ is derived from a psychological understanding of the nature of dependence and is, therefore, suitable for measuring dependence during periods of substance use or abstinence. There are 10 items scored 0-3. Cut offs are (<10 = low dependence; 10-22 = medium dependence; and >22 = high dependence).

3.10.5 Biological samples

A urine sample was collected following the interview; interview and urine sample collection together took 30-40 min. Urine samples were frozen in the laboratory at -20° C until analysis.

Ethanol levels in breath (BrAC) were collected by a clinical nurse/key worker when participants were collecting their methadone daily dose. Ethanol levels in breath (BrAC) were measured using a Lion 500 (Lion Alcolmeter) as part of routine work. Before methadone was dispensed, the clinical nurse/key worker in charge of dispensing would ask the clients to take a deep breath and expire into a disposable mouthpiece attached to the breathalyser. Readings were regularly documented in patient charts.

If the reading was above 39 mg per 100mL, methadone would not be dispensed and patients would be allowed to wait and try again subsequently. If the patient failed their breathalyser test, he or she would have to wait until 10a.m. the next day for

dispensing to begin again. If the patient failed to receive methadone for three days, he or she would need to be assessed by the consultant before being issued subsequent methadone daily doses. The scores were recorded in the participants' chart and later scanned and uploaded to the patients' journey file.

Chapter 4 EXPERIMENTS

4.1 Study (1.a) Urinalysis measurements of methadone and EDDP in methadone maintained individuals

4.1.1 Background

Using a cross-sectional prospective cohort design, Study 1 investigated the relationship between methadone and EDDP during fixed daily dosing in patients receiving methadone maintenance in order to assess whether the ratio of EDDP:methadone could be used effectively as a biomarker for methadone compliance. Four sets of results are provided: 1) the results of the descriptive statistics that summarise the basic demographic and background information on the participants; 2) the results of the t-test comparing the severity of withdrawal symptoms, alcohol drinking behavior, quality of life, methadone dosage, methadone concentration, EDDP concentration, ratio of EDDP:methadone, AUDIT score, and SOWS score between the patients in the methadone maintenance group and patients in the hazardous alcohol drinking behavior group; 3) the results of the ANOVA that was performed to determine which of the variables (methadone dosage, methadone concentration, EDDP concentration, ratio of EDDP/methadone, AUDIT score, SOWS score, and pH levels) differed significantly across the study, with measurements taken at baseline period (time 1), time 2, time 3, and time 4; and 3) the results of the correlation test that was performed in order to examine the relationship between methadone and EDDP concentration levels.

4.1.2 Recruitment and Procedures

Initial interviews and urine sample collection

Participants were asked to take part in a structured research interview involving a questionnaire that included socio-demographic questions and questions regarding licit and illicit drug use, methadone treatment, and other current treatment.

4.1.3 Data analysis

Data analysis was conducted using the Statistical Package for Social Sciences (SPSS), Windows® version 15.0. Data were collected, coded, and transferred to SPSS. The descriptive data, i.e., the demographic and background information, as well as the characteristics of licit substances and prescribed medication use, were analysed by calculating the frequencies, standard deviation, means, and percentages of participant responses. Basic demographic and background information, including patients' age, sex, ethnic background, and employment status, was compared using a chi-square test. A t-test was used to compare the continuous variables, including the severity of withdrawal symptoms (see de Wet et al., 2004). When data were not normally distributed, a non-parametric test was utilized. A Mann-Whitney U test was used to compare the distribution of continuous unpaired variables, while a Wilcoxon signed rank test was used to compare the distribution of paired variables. Data that were missing were excluded from the analysis.

4.1.4 Results

4.1.4.1 Sample Characteristics

Sixty participants at more than two weeks into methadone treatment were recruited for the study. Their mean age was 41.67 ± 8.3 years (range 23 – 56 years), and the majority were male (65%; n = 39). The ethnic background of the participants varied, but the majority described themselves as White British (58.3%; n = 35); other ethnicities were reported as follows: Other White n = 7 (11.7%), Irish n = 6 (10%), Black Caribbean n = 6 (10%), White Caribbean n = 2 (3.3%), Black African n = 2 (3.3%), Other Black (1.7%, n = 1), 'other' (1.7%, n = 1). Of the 60 participants, 38 (63.3%) reported having no formal educational qualifications, while 14 (23.3%) reported carrying a GCSE/O-Level, one (1.7%) reported having A-levels, four (6.7%) reported having vocational qualifications, and three (5%) reported having an undergraduate degree. The route of referral varied, with 26 participants (43.3%) self-referred, ten (16.7%) transferred from prison, five (8.3%) referred by a GP, two (3.3%) referred by a family member, two (3.3%) transferred from hospital, and 15 (23.3%) reporting other routes of referral. Only 13 (21.7%) of the 60 participants reported holding a driving license, while none reported driving to the clinic.

4.1.4.2 Treatment Outcome Profile (TOP)

The Treatment Outcome Profile (TOP) questionnaire records four main areas affecting the clients' life: *Substance use*: the number of days substances were used in the past 28 days; *Health*: including a participantive rating score between 0-21 of physical and psychological health status; *Crime*: the number of days when shoplifting, theft, or drug dealing occurred, as well as the prevalence of property or vehicle theft, fraud, and assault and/or other violent crime; and *Social functioning*: including a

rating of quality of life on a scale between 0 - 21 (21 being good), the number of days of paid work and attendance to education/training, period prevalence of acute housing problems, and risk of eviction.

From the TOP data section on health, crime, and social functioning, the mean physical health score was 10.28 ± 4.7 (range 1 - 19), whereas the mean score for psychological health was 9.8 ± 4.7 (range 1 - 18) and the mean score for of the quality of life (QoL) was 9.4 ± 5.1 (range 1 - 18).

When asked about problems with accommodation, 14 participants (23.3%) reported a problem with accommodation and six participants (10%) reported facing a high risk of eviction. All participants reported being unemployed, none reported receiving paid work, and only one (1.7%) reported spending days going to college. In terms of criminality, seven participants (11.7%) reported shoplifting and one reported being involved in an assault in the past 28 days.

4.1.4.3 Licit Substances

Smoking Behaviour

All 60 participants reported being current smokers. A total of 42 participants (70%) reported smoking 'roll-up' cigarettes, 12 (20%) reported smoking filtered cigarettes, and 6 (10%) reported smoking both. The mean number of cigarettes smoked per day was 13.56 ± 7.9 cigarettes (range 2 – 40 cigarettes).

Alcohol Use Behaviour

Out of the total cohort, 40 participants (66.7%) reported drinking alcohol, whereas 20 (33.3%) described themselves as non-drinkers. Among the 40 participants who

reported drinking alcohol, the mean number of units of alcohol consumed per day at baseline was 11.12 ± 8.46 (range 1 – 32) units of alcohol/day.

The participants' mean score on the Alcohol Use Disorders Identification Test (AUDIT) was 13.17 ± 13.19 (range 0 – 38 points). Out of the total cohort, 11 participants (18.3%) scored zero on the AUDIT, indicating no alcohol use in the past year. Conversely, 30 participants (50%) scored at or above the AUDIT cut-off point of ≥ 8, which suggests hazardous alcohol drinking behaviour, and the study considered these participants as a separate hazardous alcohol use (HAU) group. Notably, however, 21 of these participants (35% of the whole sample) registered AUDIT scores in the highest bracket (≥20), which identifies individuals as probable dependent drinkers. The full distribution of AUDIT scores and corresponding risk levels across the study sample is shown in Table 4-1.

Table 4-1 *Number and percent of participants at various risk levels per AUDIT scores*

Risk Level	AUDIT Score	No. of Participants	Percentage (%)
Low risk	0-7	30	50
Hazardous level	8-15	5	8.3
Harmful level	16-19	4	6.7
Dependence likely	20-40	21	35
Total		60	

AUDIT question responses identify the frequency of occurrence of a given item over the past year. Except for items one, two, nine and ten, scoring is as follows: 0 = never, 1 = less than monthly, 3 = monthly, and 4 = weekly. Table 4-1 summarises the mean scores per question on the AUDIT for sample. As these data show, 'zero' was the most prevalent response to all items except item 1. Thus, almost half of the

participants (n = 28) did not consume six or more units if female, or eight or more units if male, of alcoholic drinks on a single occasion in the last year, indicating no binging pattern in their drinking. More than half of the participants (n = 35) said that they had zero occurrences in which they were not able to stop drinking once they had started. Similarly, more than half of the participants (n = 34) said that they did not fail to do what was normally expected of them because of drinking. Nearly two-thirds (n = 39), moreover, said that they did not need an alcoholic drink in the morning to get themselves going after a heavy drinking session, and more than half (n = 33) said that they did not have a feeling of guilt or remorse after drinking. More than half (n = 33) also said that they did not experience being unable to remember what happened the night before because they had been drinking. Just over two-thirds (n = 41) said that they did not cause themselves or anyone else to be injured as a result of their drinking, and more than half (n = 32) said that they did not have a relative or friend or a doctor or another health worker express concern about their drinking or suggest that they cut down. Nonetheless, the extent of participants' drinking behaviour should not be minimised, as the same data show that almost half of the participants (n = 28) *did* have people concerned about their drinking, and nearly one-third (19) *had* injured themselves or others (see Table 4-2).

Table 4-2 *Distribution and mean of AUDIT item responses across samples*

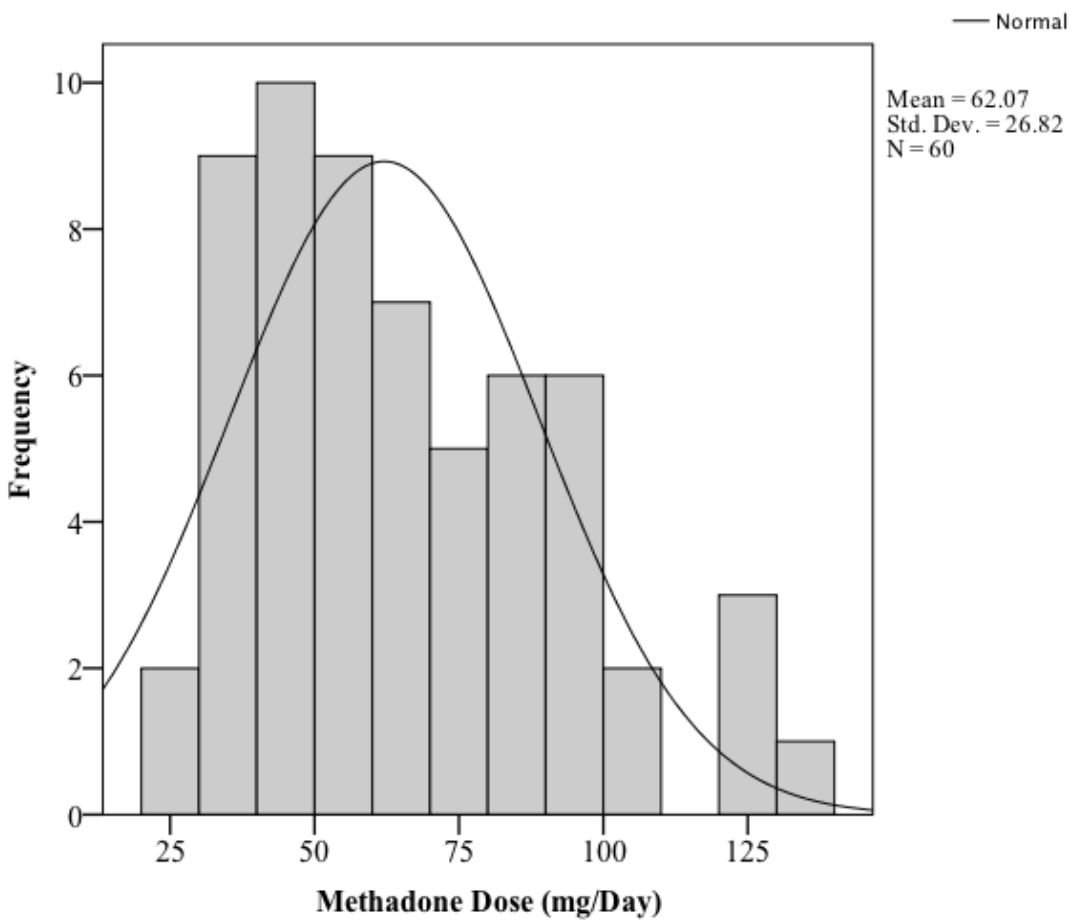
AUDIT question	0 points	1 point	2 points	3 points	4 points	Mean
How often do you have a drink containing alcohol?	10	10	6	7	27	2.52
How many units of alcohol do you drink on a typical day when you are drinking?	33	10	7	4	6	1.27
How often have you had 6 or more units if female, or 8 or more if male, on a single occasion in the last year?	28	8	4	7	13	1.48
How often during the last year have you found that you were not able to stop drinking once you had started?	35	4	3	4	14	1.3
How often during the last year have you failed to do what was normally expected from you because of drinking?	34	6	8	8	4	1.03
How often during the last year have you needed an alcoholic drink in the morning to get yourself going after a heavy drinking session?	39	3	3	5	10	1.06
How often during the last year have you had a feeling of guilt or remorse after drinking?	33	3	2	11	11	1.4
How often during the last year have you been unable to remember what happened the night before because you had been drinking?	33	10	8	6	3	0.93
Have you or someone else been injured as a result of your drinking?	41	1	5	0	13	1.05
Has a relative or friend or a doctor or another health worker been concerned about your drinking or suggested you cut down?	32	0	3	1	24	1.75

There is also a shorter version of the Alcohol Use Disorder Identification Test (AUDIT-C) that takes into account only the first three questions of the AUDIT. The mean AUDIT-C score for the sample was 4.9 ± 4 (range 0 – 12).

Methadone Treatment

All participants were prescribed a fixed daily dose of methadone from the community drug service. The mean dose for the whole sample at baseline was 62 ± 26.8 mg methadone/day (range 25 - 130 mg methadone/day) and the median was 55 mg of methadone/day. The most frequent dose prescribed by the clinic to participants was 50 mg methadone/day (n = 9; 15%), as can be seen in Figure 6-1. In addition to the prescription data summarised in this figure, seventeen participants (28.3%) reported using methadone on top of their prescribed daily dose.

Figure 4-1 *Daily methadone dose prescribed to participants in terms of frequency (number of participants) across the sample*



In terms of adherence to methadone dosing, twenty-seven participants (45%) reported missing their daily methadone dose in the previous seven days, while 21 (35%) reported never missing their daily methadone dose. Of those who missed their daily dose, the most common reason stated was arriving late to the clinic (n = 15; 25%). Eleven participants (18.3%) reported that this was related to alcohol use, which could include being drunk, knowing that they would exceed the breathalyser cut off limit, or being unconscious. Four participants (6.7%) reported missing their dose to be related to a police arrest and another four reported being too unwell to attend.

4.1.4.4 Subjective Opiate Withdrawal Scale (SOWS)

In interviews that took place during trough, i.e., at the end of the dosing interval, before the next subsequent dose, participants were also asked to complete the Subjective Opiate Withdrawal Scale (SOWS). This tool measures the presence and intensity of symptoms of opiate/opioid withdrawal, including musculoskeletal, psychiatric, autonomic, gastrointestinal, and motor signs, from the patient's perspective. The SOWS is a self-administered ten-item self-report questionnaire on which each item is rated on a 4-point scale (0 = none, 1 = mild, 2 = moderate, 3 = severe) (see Table 6-3). The mean SOWS score for the whole sample was 5.1 ± 5.9 (range 0 – 27).

Table 6-3 shows the distribution of participants' responses to the ten SOWS items, which provides a picture of their withdrawal experience. Overall, 'none' was the most common response to all items, with 'mild' next frequent for items 1-5 (except item 3), and 'moderate' receiving a similar or greater number of responses as 'mild' for items 3 and 6-10. By far the most commonly cited as 'severe' (n = 11) was the symptom *insomnia*, which was also the symptom that received the fewest responses

of 'none' (n = 30).

Table 4-3 *Distribution and mean scores of SOWS item responses across the sample*

	Number of participants scoring at each level				
SOWS item	**None (0)**	**Mild (1)**	**Moderate (2)**	**Severe (3)**	**Mean Total score**
1.Feeling Sick	41	10	6	3	0.51
2.Stomach cramps	45	6	6	5	0.45
3.Muscle spasms	50	3	5	2	0.31
4.Feelings of coldness	36	13	7	4	0.65
5.Heart pounding	47	6	4	3	0.38
6.Muscular tension	44	5	9	2	0.48
7.Aches and pains	39	8	9	4	0.63
8. Yawning	38	11	8	3	0.6
9.Runny eyes	43	6	9	2	0.5
10.Insomnia	30	9	10	11	1.1

Additionally, a Pearson Correlation coefficient was conducted to determine the strength of the relationship, if any, among overall SOWS and two other variables: cigarette usage and methadone dose. As can be seen in Table 4-4 below, the results showed the frequency of cigarette usage, size of methadone dose, and the SOWS score for each participant. A Pearson Correlation coefficient revealed that the frequency of cigarette smoking with the mean of 13.56 ± 7.98 and SOWS score with the mean of 5.57 ± 6.79 were not correlated (r = 0.07, n = 60, df = 58, p = 0.59). There was no correlation between the size of methadone dose with the mean of 62.07 ± 26.82 and frequency of cigarette smoking either (r = 0.02, n = 60, df = 58, p = 0.86).

Table 4-4 ***Table use, methadone dose, and SOWS scores by participant***
Illicit Drug Use

Participant number	SOWS score	Methadone Dose (mg)	Cigarettes/ Day	Participant number	SOWS score	Methadone Dose (mg)	Cigarettes/ Day
1	0	45	15	31	16	40	25
2	0	40	6	32	5	95	40
3	0	80	2	33	0	65	15
4	0	70	12	34	0	120	3
5	1	30	6	35	25	50	11
6	0	30	12	36	2	85	13
7	0	40	14	37	7	100	14
8	14	50	10	38	11	130	10
9	1	40	15	39	17	80	20
10	6	95	20	40	15	35	8
11	4	90	15	41	8	50	10
12	9	30	20	42	16	60	35
13	1	50	15	43	11	70	3
14	15	25	15	44	6	60	20
15	8	50	10	45	23	30	13
16	7	74	5	46	2	45	5
17	4	25	10	47	6	70	10
18	0	50	10	48	8	80	13
19	3	50	3	49	0	35	17
20	1	120	3	50	2	50	10
21	0	30	2	51	4	30	10
22	2	90	20	52	3	80	10
23	0	35	10	53	5	90	20
24	3	85	35	54	2	65	20
25	0	70	20	55	2	40	30
26	3	95	10	56	7	100	8
27	0	40	15	57	0	45	20
28	0	45	15	58	5	60	6
29	27	120	10	59	0	60	10
30	0	60	20	60	17	50	10
				Mean	13.5	62	5.5
				± SD	±7.8	± 26.8	± 6.7

Data on drug use in the 28 days prior to the interview were collected using the TOP. Forty-three participants (72.7%) self-reported that they used heroin, and 41 (68.3%) reported using crack cocaine on top of their prescribed opioid. Use of powdered cocaine was reported only by one participant. Twenty-eight participants (46.7%) reported using cannabis, and nine (15%) reported using illicit benzodiazepines. One participant (1.7%) used amphetamines, while four (6.7%) reported using other forms of drugs illicitly. A total of 25 participants (41.7%) self-reported drug use by the intravenous route in the past 28 days.

4.1.4.5 Prescribed Medication

Thirty-six participants (60%) reported being prescribed another medication along with their methadone; 12 participants (20%) received more than two medications, eight (13.3%) were prescribed antidepressants, four (6.7%) were prescribed anxiolytics, three (5%) were prescribed antipsychotics, and nine (15%) reported being prescribed other medications.

4.1.4.6 Gender, Sample Characteristics, and Survey-Measured Variables

Tables 4-5, 4-6, and 4-7 show, respectively, the characteristics *ethnicity* and *route of referral* and the demographic variable *level of education* as self-reported by the 39 male and 21 female participants in the study. The mean age for the male participants was 44 ± 7.9 years compared to 38 ± 7.9 for female participants. Independent t-test indicated significant difference between the genders by age ($t = -2.63$, $df = 58$, $p = 0.01$) with male participants slightly older than female participants. However, a Chi-Square test indicated p-values for these variables were all greater than the level of

significance, indicating that there were no significant relationships by gender for the demographic characteristics.

Table 4-5 *Ethnicity of Participants*

Demographic	Male (n=39)	Female (n=21)
White British	23	12
White Caribbean	1	1
Other Black	0	1
Irish	4	2
Caribbean	6	0
Other White	3	4
African	1	1
Other	1	0
Chi-Square	7.54	
df	7	
p-value	0.37	

Table 4-6 *Route of Referral for Participants*

Route of Referral	Male (n=39)	Female (n=21)
Came by self	19	7
Transfer from hospital	1	1
Referred by GP	2	3
Sent by family	2	2
Transfer from prison	6	2
Referred by a consultant	1	0
Other	8	6
Chi-Square	3.98	
df	6	
p-value	0.67	

Table 4-7 *Education Level of Participants*

Qualifications obtained	Male (n=39)	Female (n=21)
No formal qualifications	27	11
GCSE / O-Level	6	8
A-Level	1	0
Vocational qualifications (e.g. HND, NVQ)	4	0
Undergraduate Degree	1	2
Chi-Square	7.64	
df	4	
p-value	0.95	

Table 4-8 summarises the mean scores of the survey's self-reported variables, i.e, the socio-demographic information, health variables, previous drug use, treatments received, AUDIT scores, SOWS scores, and so on, for male and female participants. Again, independent t-test showed no significant differences by gender for these variables.

Table 4-8 *Mean scores for health, alcohol use, methadone treatment, withdrawal symptons and illict drug use*

Variable	Male (n=39)	Female (n=21)	t	df	p-value
Physical Health Score	10.51	8.83	-1.69	58	0.50
Psychological Health Score	11.03	8.90	-1.69	58	0.45
Quality Life Score	10.38	7.60	-2.07	58	0.56
Alcohol use	25	15	-.056	58	0.60
Units/day	12.98	10.92	0.29	58	0.65
AUDIT score	12.92	13.62	0.19	58	0.84
Cigarette use/day	12.68	25.29	1.17	58	0.50
Methadone dose (mean)	60	65	0.67	58	0.56
Methadone use on top (yes/no)	10	7	-0.67	58	0.78
SOWS score	5.74	5.24	-0.27	58	0.78
Illicit heroin use	25	16	0.03	58	0.65
Crack cocaine	38	21	-0.95	58	0.32
Cannabis	20	8	0.97	58	0.90
Illicit Benzodiazepine	6	3	0.11	58	0.90
Intravenous use	17	8	0.41	58	0.68

A comparison of the opiate withdrawal scores at baseline between female with a mean of 5.24 ± 5.33 and male with the mean score of 5.74 ± 7.51 participants was also conducted using independent t-test, and the results showed no significant differences ($t = -0.27$, $df = 58$, $p = 0.78$). Table 4-9 summarises the item-by-item SOWS scores at baseline divided among the female and male patients as well as the p-value derived from the t-test that was conducted to examine gender differences. No statistically significant difference between male and female responses was identified.

Table 4-9 *SOWS item responses across sample by gender*

	Number of participants scoring at each severity level							
SOWS question	**None (0)**	**Mild (1)**	**Moder -ate (2)**	**Severe (3)**	**Mean Total score**	**t**	**df**	**p-value**
	M/F	**M/F**	**M/F**	**M/F**	**M/F**			
1.Feeling Sick	27/14	7/3	3/3	2/1	0.48/0.57	0.35	58	0.73
2.Stomach cramps	30/15	4/2	3/3	2/1	0.41/0.52	0.43	58	0.63
3.Muscle spasms	33/17	2/1	2/3	2/0	0.30/0.33	0.90	58	0.90
4.Feelings of coldness	23/13	8/5	5/2	3/1	0.69/0.57	0.43	58	0.64
5.Heart pounding	32/15	3/3	2/2	2/1	0.33/0.47	0.39	58	0.53
6.Muscular tension	28/16	3/2	5/3	2/0	0.53/0.38	0.16	58	0.51
7.Aches and pains	26/13	4/4	6/3	3/1	0.64/0.61	0.53	58	0.93
8. Yawning	24/14	8/3	5/2	2/1	0.61/0.57	0.97	58	0.86
9.Runny eyes	27/16	3/3	7/2	2/0	0.58/0.33	0.02	58	0.28
10.Insomnia	19/11	7/2	3/7	10/1	1.10/0.90	0.27	58	0.55

4.1.4.7 Biological Measures

Urine samples were collected at baseline (period 1) from 60 participants (4 samples were discarded). Three further urine samples were collected at one-week intervals, with 35 participants providing samples during period 2, 30 during period 3, and 17 during period 4, for a total of 138 samples collected. All samples were analysed for methadone and its primary metabolite, EDDP, using High Performance Liquid Chromatography.

Table 4-10 *Summary of Number of Samples Collected by Data Collection Period*

Period of collection	**Number of samples collected**
Baseline (1)	56
2	35
3	30
4	17
Total urine samples collected	138

As a group, 21 participants (35%) provided one sample, five (8.3%) provided two samples, 13 (20%) provided three samples, and 17 (30%) provided four samples. Four participants provided less than 2 mL of urine at baseline, which hindered the analysis.

Urinary methadone and EDDP concentration range values

The mean urinary methadone concentration at trough for the sample was 3497.23 ± 4553.76 µg/L (5.90 – 20351.8) and the mean urinary EDDP concentration at trough was 15106.95 ± 36208.33 µg/L (0.05 – 302940). To control variation, the samples were arranged according to methadone dose as reported by the participants. Figure 4-2 shows a scatter plot of the methadone and EDDP concentrations in urine in relation to daily methadone dose. There are three outliers, which are excluded in further consideration of these data.

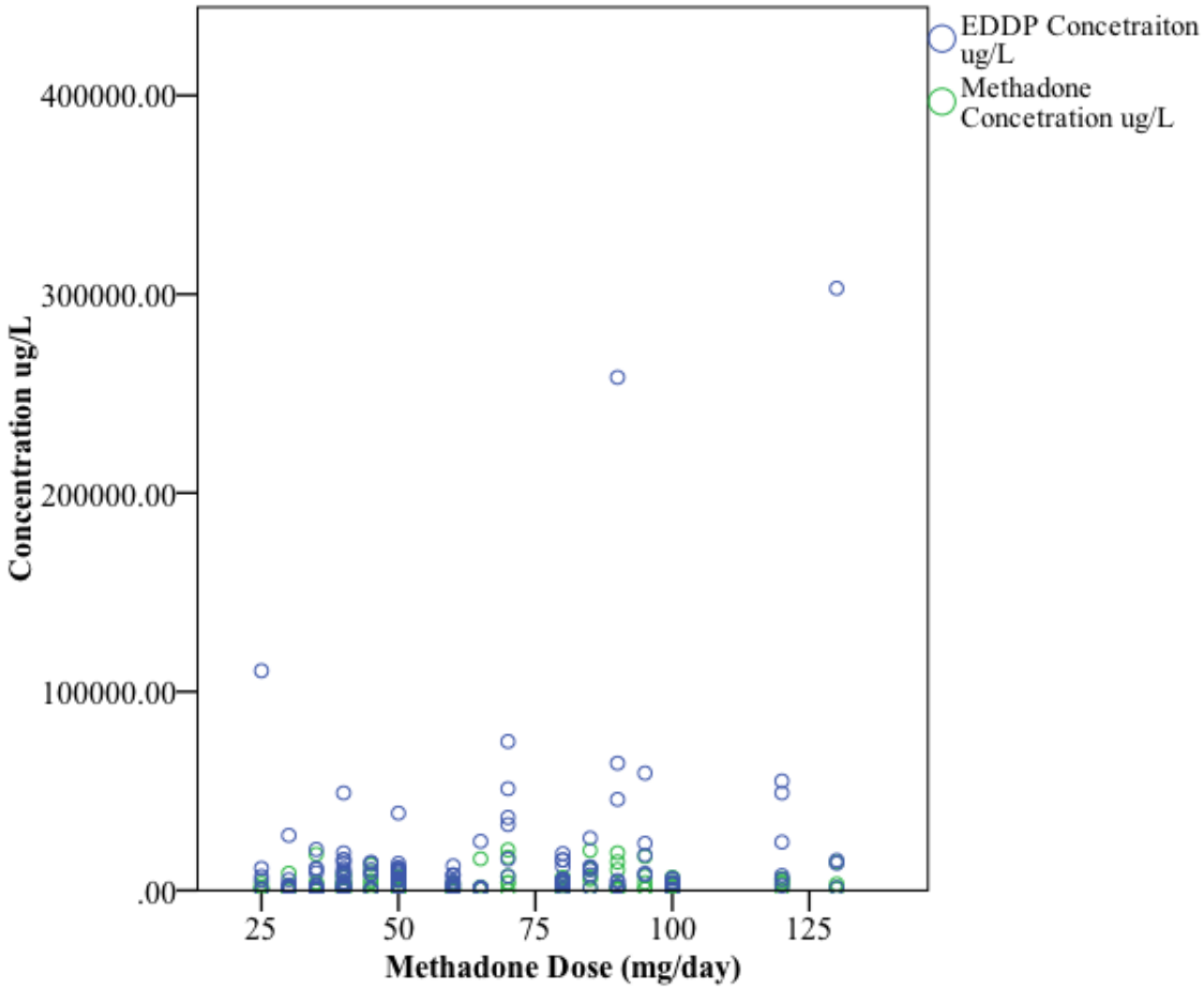


Figure 4-2 *Scatter plot representing methadone (green hollow circles) and EDDP concentration (blue hollow circles) against daily methadone dose*.

Table 4-11 summarises the distribution of methadone and EDDP urine concentrations by methadone dose.

Table 4-11 *Urinary methadone and EDDP concentrations by methadone dose*

Methadone Dose (mg)	No. Participants on dose	No urine samples included	Average Methadone Conc µg/L	Average EDDP Conc µg/L	EDDP: methadone EDDP Ratio	Methadone: EDDP Ratio
25	2	5	1426.71	8585.83	6.02	0.17
30	5	9	1571.85	2524.33	1.61	0.62
35	3	12	2381.78	3268.83	1.37	0.73
40	6	18*	2782.73	9432.21	3.39	0.30
45	4	7	4794.79	3222.33	0.67	1.49
50	9*	16	2436.58	8583.89	3.52	0.28
60	5	12	1073.68	2886.89	2.69	0.37
65	2	5	3976.72	1608.72	0.40	2.47
70	4	6	8670.57	11757.28	1.36	0.74
80	4	12	2519.08	4056.67	1.61	0.62
85	2	6	9228.51	3858.17	0.42	2.39
90	3	8	6042.78	6323.22	1.05	0.96
95	3	5	6003.96	6738.67	1.12	0.89
100	1	7	2660.31	1681.94	0.63	1.58
120	3	6	3857.81	7854.17	2.04	0.49
130	1	4	4893.24	32970.94	6.74	0.15

A Pearson correlation test was conducted to determine the relationship between the following variables: methadone dosage, methadone concentration, EDDP concentration, and ratio of methadone/EDDP. The results showed that the methadone concentration was significantly positively correlated with methadone dosage ($r = 0.22$, $df = 136$, $p = 0.01$) and with EDDP concentration ($r = 0.24$, $df = 136$, $p<0.001$). The results of the correlation test also showed that the methadone concentration was significantly positive correlated with the EDDP concentration ($r = 0.39$, $df = 136$ $p<0.001$). This means that the concentration of methadone and EDDP increase when

methadone dose increases. However, no significant relationship was noted between methadone or EDDP and the metabolic ratio (EDDP:methadone).

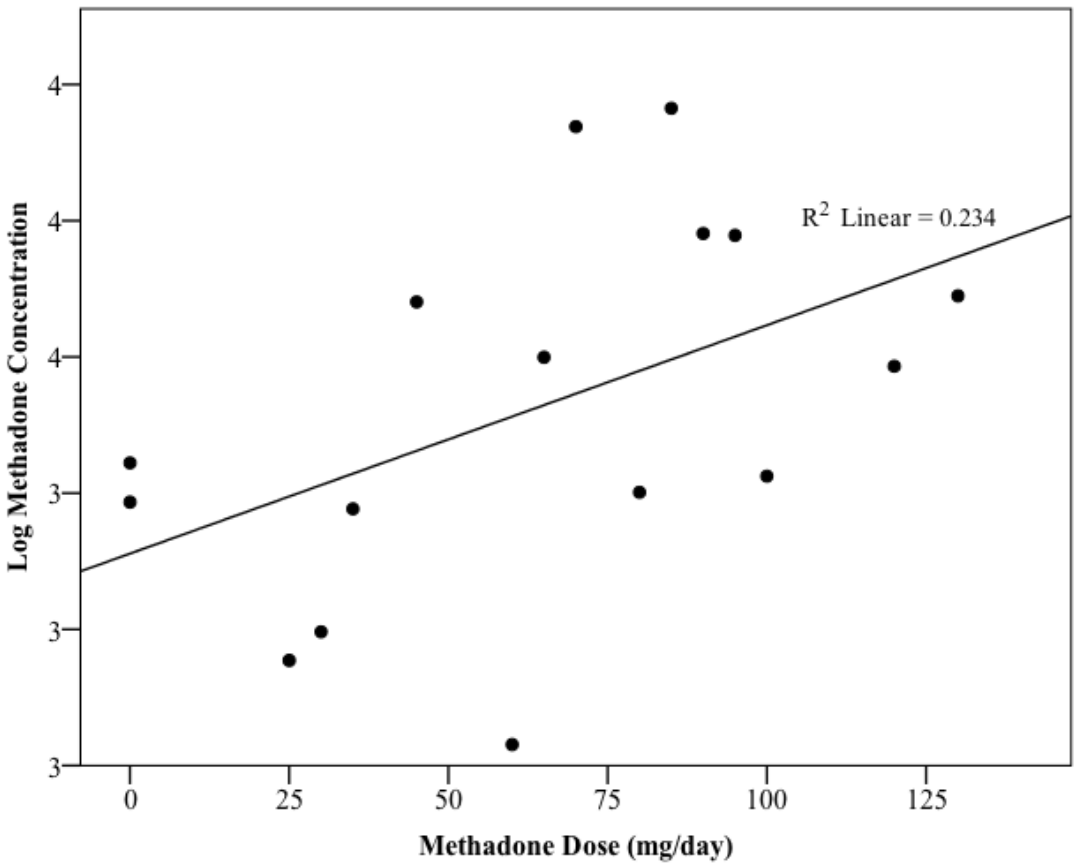


Figure 4-3 *Scatterplot of log methadone concentration against methadone dose*.

Figure 4-3 shows the relationship between methadone dose and average methadone concentration. The graph demonstrates an increasing trend, which indicates a significant positive relationship between methadone dose and methadone concentration.

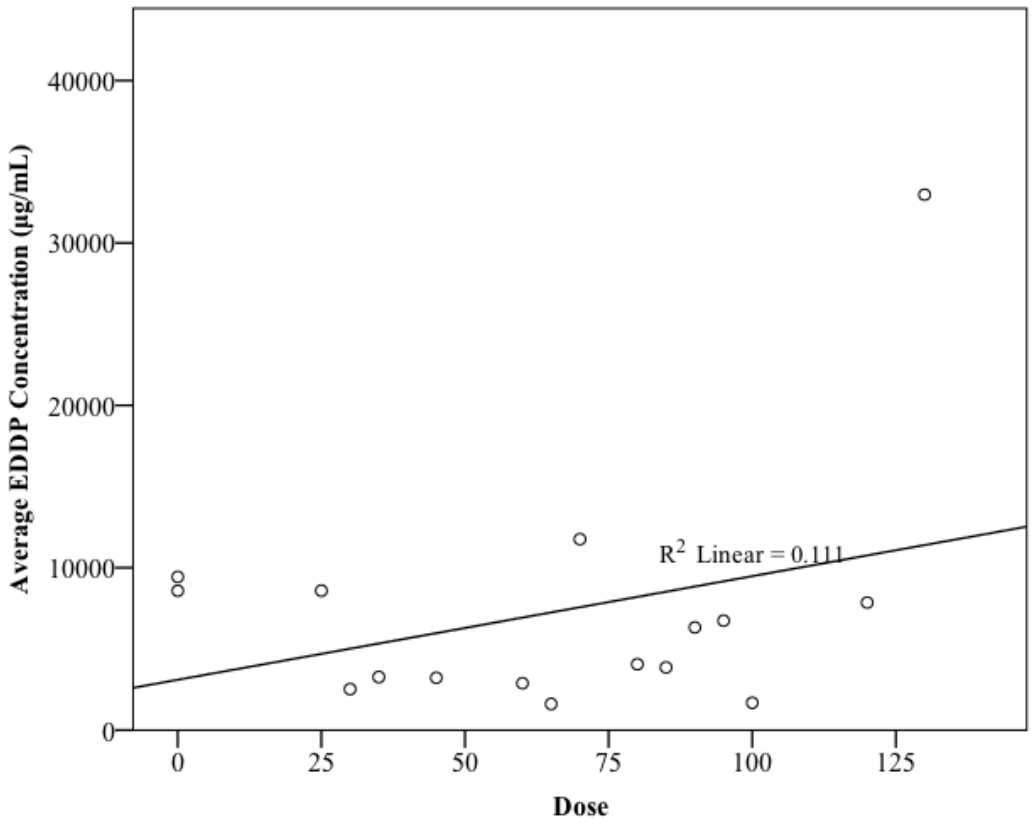


Figure 4-4 *Scatterplot of Methadone Dose against Average EDDP concentration*

There was a significant correlation between methadone concentration and the AUDIT Scores ($r = -0.16$, $df = 136$, $p = 0.05$). However, methadone concentration was not significantly correlated with SOWS scores ($r = -0.12$, $df = 136$, $p = 0.18$) or between EDDP concentration and either the AUDIT Scores ($r = -0.13$, $df = 136$, $p = 0.12$) or SOWS scores ($r = -0.03$, $df = 136$, $p = 0.65$). A significant positive correlation, however, was observed for methadone dosage and AUDIT scores ($r = 0.23$, $df = 136$, $p = 0.01$). Also a significant positive correlation was observed with the AUDIT score and SOWS score ($r = 0.25$, $df = 136$, $p<0.001$).

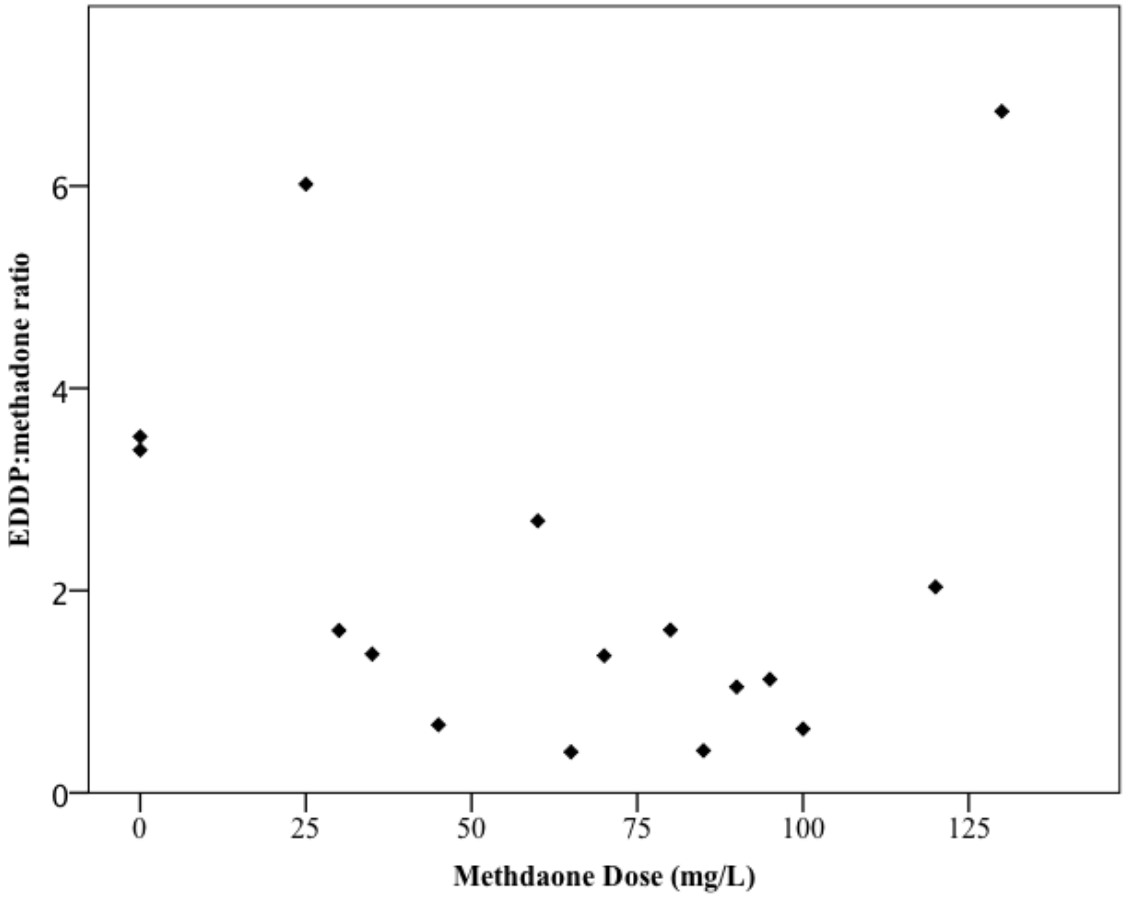


Figure 4-5 *Scatter plot of mean EDDP : methadone ratio (blacksquares) against methadone dose (mg/L)*

Results of the Pearson Correlation coefficient showed that ratio of EDDP:methadone 19.05 ± 54.21 and methadone dosage 63.19 ± 27.78 were not correlated ($r = -0.05$, $df = 136$, $p = 0.96$) (see Figure 4-5). However, methadone concentration was negitavely correlated with pH level ($r = -0.37$, $df = 136$, $p<0.001$,) see figure (4-6).

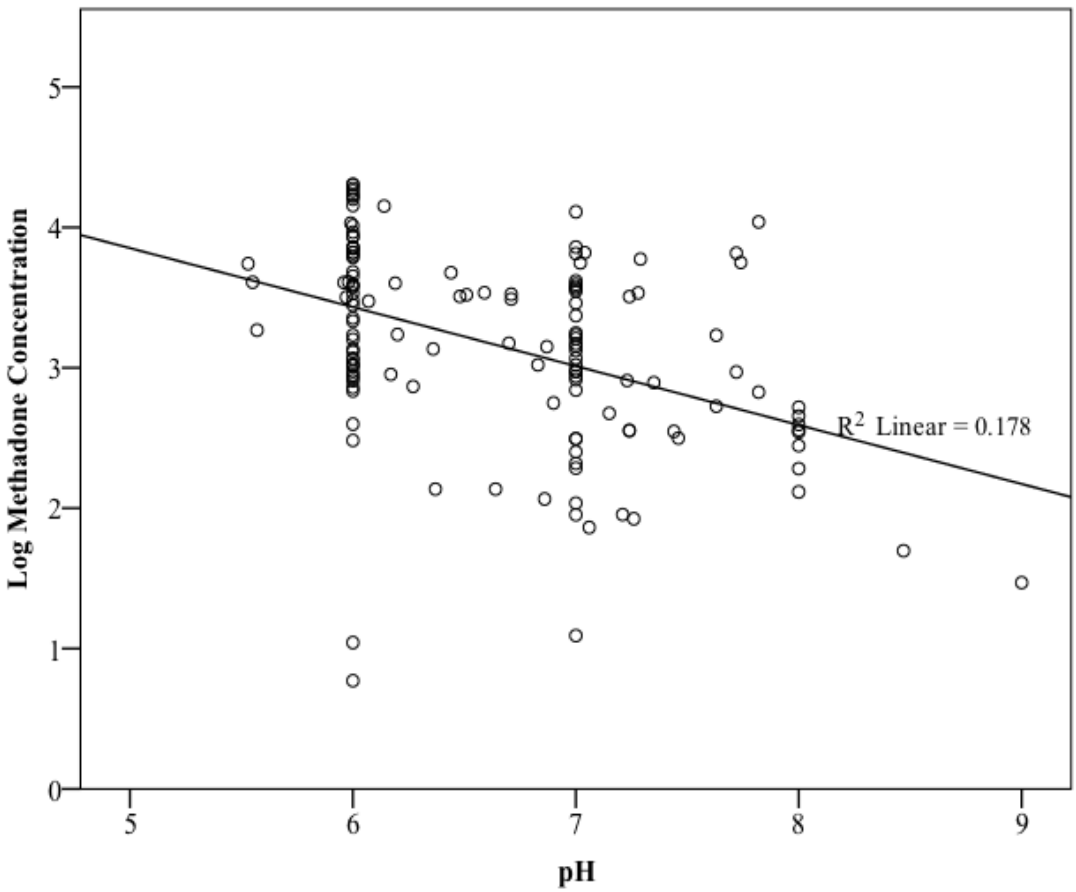


Figure 4-6 *Scatterplot of Log methadone concentration against pH*

Between-patient relationship for dose and urinary methadone concentration

Of the 138 samples collected, the mean dose was 63.1 ± 27.7 mg/day (25 - 130) and the median dose was 60 mg/day. However, the most common dose linked to the urine samples was 40 mg/day (n = 18; 7.5%). Table 4-12 summarises the participants' methadone and EDDP urine concentrations, ratios, and pH at this dose. Mean concentration of methadone was 2782.73 µg/L and mean EDDP was 9649.8 µg/L.

Table 4-12 *Methadone and EDDP urine concentrations in samples at the most commonly prescribed methadone dose for the sample (40mg/day)*

Sample Number	pH	Methadone Con (μg/L)	EDDP Con (μg/L)	EDDP: methadone ratio	Methadone: EDDP ratio
1	6	7219.9	15512	2.14	0.46
2	7.28	3413.55	15349	4.49	0.22
3	8	394.52	4252	10.77	0.09
4	6	1362.72	1559	1.14	0.87
5	7.72	933.01	1657	1.77	0.56
6	6	3864.1	2131	0.55	1.81
7	5.97	3184.99	6318	1.98	0.50
8	7.63	1706.27	9390	5.5	0.18
9	6	3762.69	9116	2.42	0.41
10	7.72	8.38	1196	142.69	0.0
11*	7	954.9	49052	51.36	0.01
12	6.71	3084.29	10973	3.55	0.28
13	6	8572.63	7688	0.89	1.11
14	6.87	1413.39	2089	1.47	0.67
15	7	312.34	981	3.14	0.31
16	6.71	3345.41	13592	4.06	0.24
17	8.47	49.74	3968	79.76	0.01
18	7	6506.3	18875	2.9	0.34
Mean ±SD	6.8 ± 0.7	2782.7 ± 2531.6	9649.8 ± 11345.2	17.8 ± 37.4	0.4 ± 0.45

*Sample 11 was identified as an outlier and is excluded from the graphic presentation in Figure 4-7

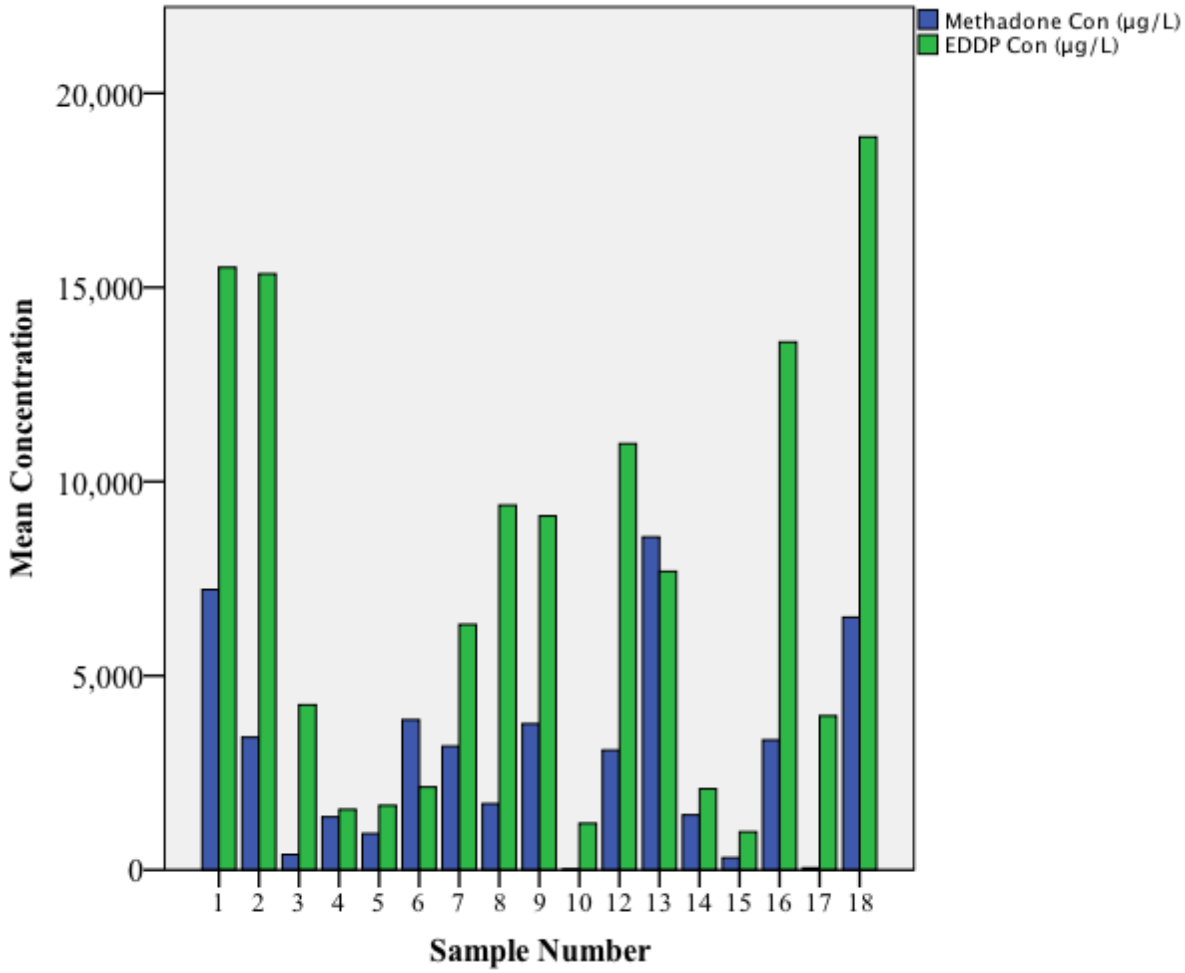


Figure 4-7 *Distribution of methadone concentrations (blue bar) and EDDP concentrations (green bar) in 18 samples from participants receiving a dose of 40 mg/day of methadone*.

As seen in Figure 4-7, EDDP concentrations were higher than methadone concentrations in all of the 40mg/day samples except for numbers 6 and 13. The mean EDDP:methadone ratio was 17.8 ± 37.48 (0.5 - 142.6), with a variance of 1405.1. This result showed a high variability in the ratio among patients receiving the same dose.

Similarly, 16 samples were collected from participants on a 50mg/day methadone dose. The concentrations, ratios, and pH for these samples are summarised in Table 4-13 below.

Table 4-13 *Methadone and EDDP urine concentration in samples from participants with methadone dose of 50 mg/day*

Sample Number	pH	Methadone Con (μg/L)	EDDP Con (μg/L)	EDDP: methadone Ratio	Methadone: EDDP Ratio
1	8	453.4	5539	12.22	0.082
2	7	1421.67	9410	6.62	0.151
3	7.46	315.66	1328	4.21	0.238
4	6	6117.17	8271	1.35	0.74
5*	8	348.67	10943	31.38	0.032
6	7.44	352.11	5046	14.33	0.07
7	6.44	4760.12	11566	2.43	0.412
8	7	1618.53	9675	5.98	0.167
9	6	6278.74	13582	2.16	0.462
10	6	1713.33	9403	5.49	0.182
11	6	7079.14	38878	5.49	0.182
12	7	1770.88	5579	3.15	0.317
13	5.57	1854.09	2189	1.18	0.847
14	7	1048.47	10001	9.54	0.105
15	6.7	1498.03	2705	1.81	0.554
16	7	2355.23	10395	4.41	0.227
Mean ±SD	6.78 ± 0.73	2436.57 ± 2283.5	9656.87 ± 8585.27	6.98 ± 7.545	0.29 ± 0.24

*Outlier

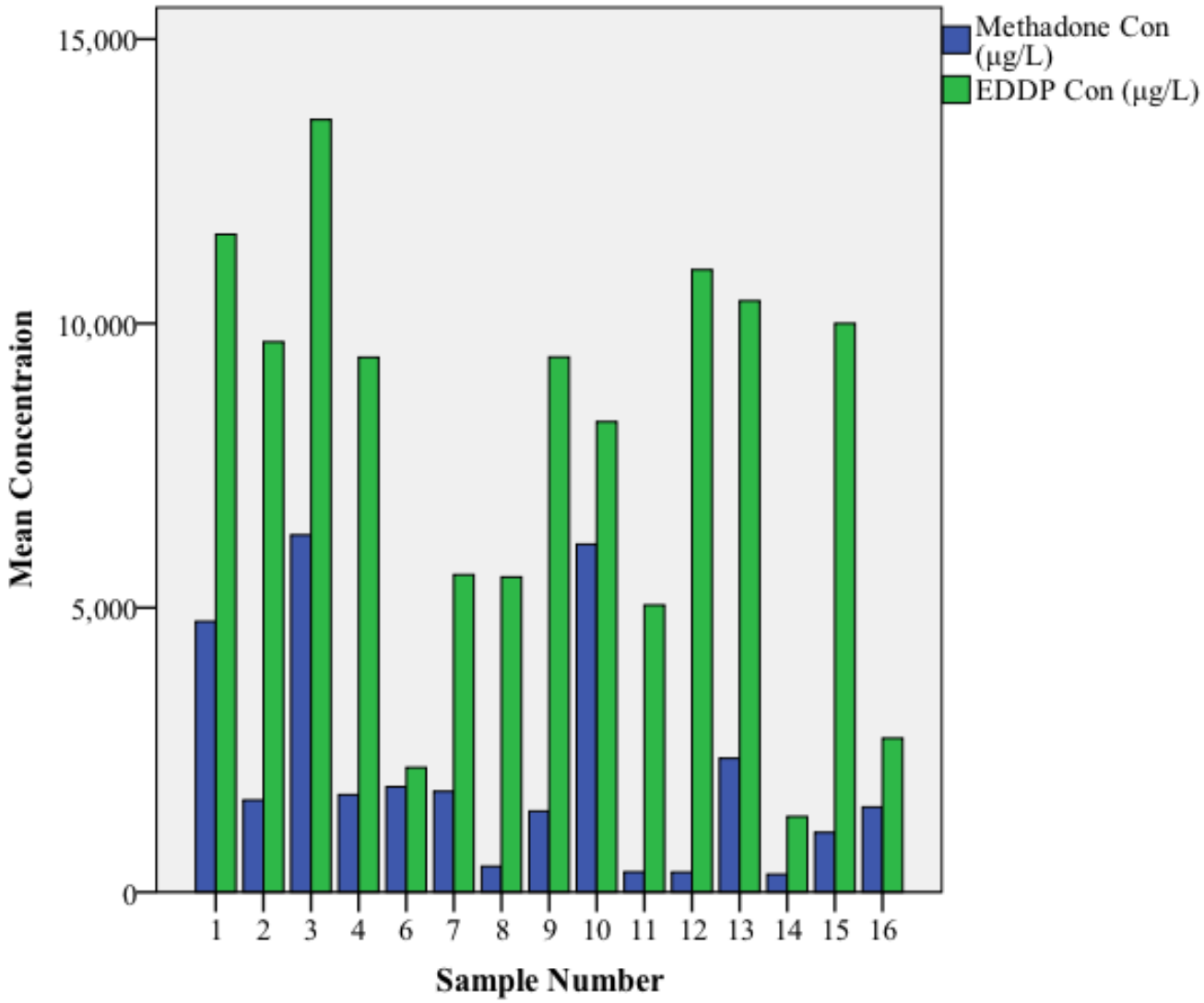


Figure 4-8 *Distribution of methadone concentration (blue bar) and EDDP concentrations (green bar) in 18 samples from participants receiving a dose of 50 mg/day of methadone*.

As seen in Figure 4-8, EDDP concentrations were higher than methadone concentrations in all of the 50mg/day samples. The mean EDDP:methadone ratio was 6.9 7.5 (1.1 – 31.3). The variance was 56.9, which is less than was found in the samples collected from participants receiving a 40mg/day methadone dose.

Comparison of male and female participants' urinary methadone and EDDP concentrations

As there were differences in the male and female participants in this study with regard to methadone dose and alcohol consumption, the methadone concentrations in their urine samples were also considered separately. Table 4-14 summarises the concentrations and ratios by gender.

Table 4-14 *Dose vs average urinary methadone and EDDP concentration frequency across the sample by gender*

Gender	No. of samples examined	Average Methadone Conc µg/L	Average EDDP Conc µg/L	Methadone: EDDP Ratio	EDDP: methadone Ratio
Male (n=39)	100	3187.21	10503.45	20.04	1.72
Female(n=2)	38	4311.95	27209.06	16.43	0.44
t		-1.3	-2.55	0.34	0.67
df		136	39	136	136
p-value		0.19	0.11	0.72	0.56

Using an independent t-test, results indicated that there was no significant difference between males and females in methadone, EDDP concentration, or Methadone EDDP ratio. EDDP:methadone ratio also did not indicate significant difference between male and female. Figure 4-9 presents the differences graphically between methadone and EDDP concentrations in male and females.

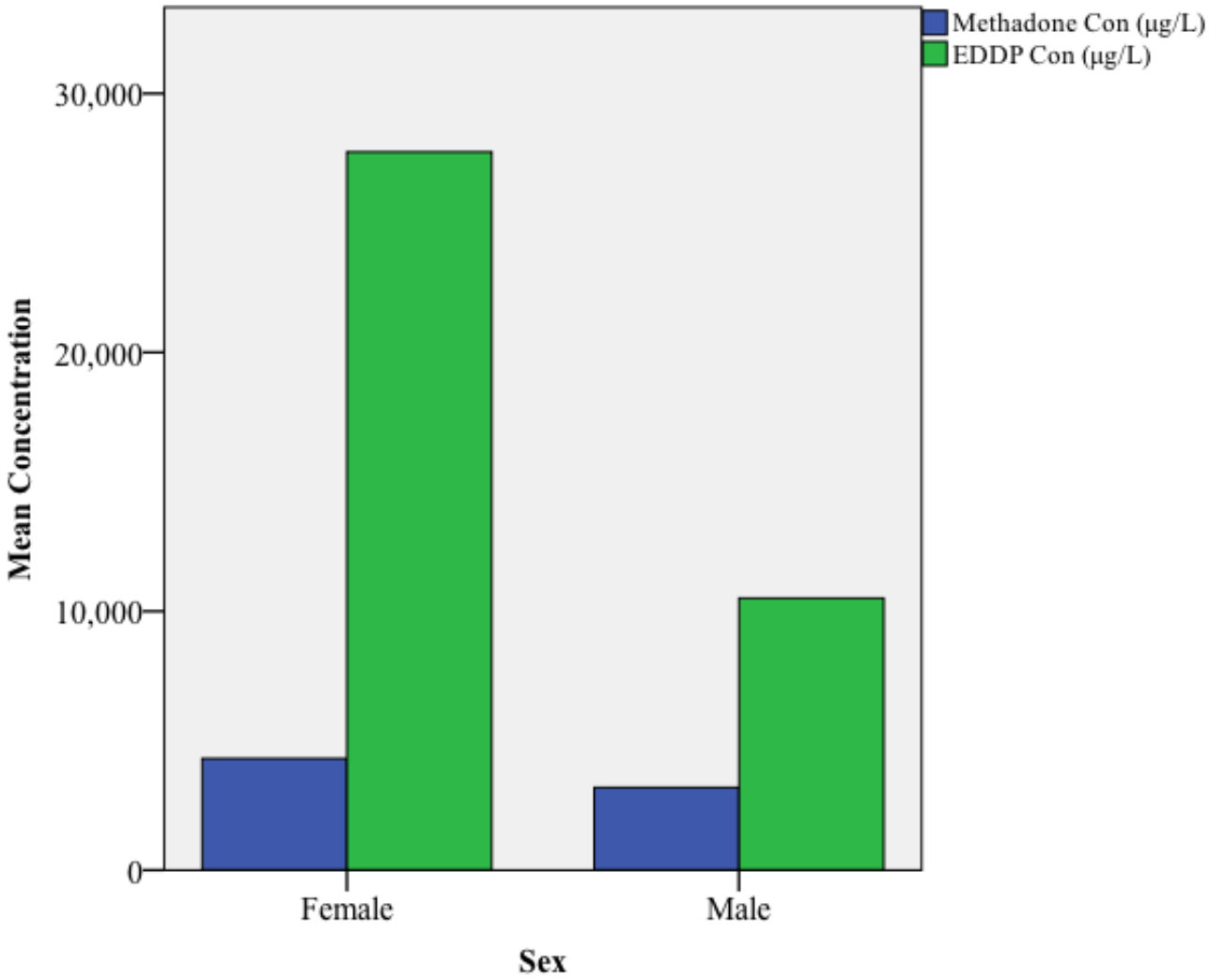


Figure 4-9 *Differences in methadone (blue bar) and EDDP concentration (green bar) between males and females across all samples collected (138)*

4.1.4.8 Methadone Maintenance Group (MMT) versus Hazardous Alcohol Use Behaviour Group (HAU)

A comparison was undertaken between participants who had AUDIT scores at or above the cut-off (≥8) for hazardous drinking (i.e., the hazardous alcohol use behaviour group, HAU) and the remainder of the sample (methadone maintenance group, MMT) (n = 30; n = 30).

Sample Characteristics and Demographic Data

The mean age of the HAU group was 41.1 ± 7.7 years (range 26 - 56 years). The ethnic background was varied in both groups; however, the HAU group contained a slightly higher proportion of White British participants (70%, n = 21) as shown in Table 4-17. A total of 5 HAU participants (16.7%) reported having completed GCSEs/O-Levels, fewer (6.7%, n = 2) had received a vocational qualification and (6.7%, n = 2) had an undergraduate degree. About two-thirds (60%, n = 18) did not have any formal qualifications. The route of referral varied, but half reported being self-referred (50%, n = 15). Five participants (16.7%) were referred by a general practitioner, three (10%) transferred from prison, one (3.3%) was sent by their family, one (3.3%) reported being transferred from hospital, and one (3.3%) was referred by a consultant. Four participants (13.3%) reported other routes of referral (see Table 4-15).

Methadone Maintenance Group

A total of 30 participants of which (70%, n = 21) were male scored ≤8 on the AUDIT. The mean age in this group was 42.2 ± 8.9 years (range 23 - 56 years). The ethnic background of the participants varied but majority described themselves as White British (46%, n = 14) while other ethnicities were reported in smaller numbers: Caribbean (20%, n = 6), White Caribbean (6.7%, n = 2), Irish (10% n = 3), and Other White (10%, n = 3) (see Tables 4-16 and 4-17).

Table 4-15 *Summary of comparative demographic data for methadone maintenance and hazardous alcohol use groups – referral source and education*

Demographic parameter	MMT Group	HAU Group
Referral source		
Transfer from hospital	1	1
Referred by GP	0	5
Sent by family	3	1
Transfer from prison	5	3
Referred by a consultant	0	1
Other	10	4
Came by self	11	15
Chi-Square	10.69	
df	6	
p-value	0.09	
Education		
No formal qualifications	18	20
Chi-Square	2.58	
df	4	
p-value	0.63	

A total of 9 participants (30%) reported having completed GCSEs/O-Levels, fewer (6.7%, n = 7) had received an occupational training and only one participant (3.3%) had an undergraduate degree. More than half (60%, n = 18) did not receive any formal qualifications. The route of referral varied; (36.7, n = 11%) were self-referred, five participants (16.7%) reported being transferred from prison. Only three (10%) were sent by their family and one (3.3%) reported being transferred from hospital. Ten participants (33.3%) were referred by other routes (Table 4-15). A Chi-Square test was conducted and found no significant relationship in all the parameters between the two groups. An independent t-test indicated no significant difference between the two groups in the age ($t = -0.5$, $df = 58$, $p = 0.62$).

Table 4-16 *A summary of the comparative demographics data for MMT and HAU groups*

Demographic parameter	MMT Group	HAU Group
Sex		
Male	21	18
Female	9	12
t	0.49	
df	58	
p-value	0.29	

Table 4-17 *A summary of the comparative demographic data for MMT and HAU groups in terms of ethnicity*

Demographic parameter	MMT Group	HAU Group
Ethnicity		
White British	14	21
White Caribbean	2	0
Other Black	0	1
Irish	3	3
Caribbean	6	0
Other White	3	4
African	1	1
Other	1	0
Chi-Square	7.55	
df	7	
P value	0.11	

4.1.4.8.1.1 Treatment Outcome Profile (TOP)

According to the TOP data, the mean physical health score of the HAU group was 10.7 whereas the mean score for psychological health was 9.57. The mean score for

the quality of life (QoL) was 5.15. Using t-test analysis there were no differences in the scores when compared to MMT group. Table 4-18 summarises the data.

Table 4-18 *A summary of the comparative demographics data for MMT and HAU groups in terms of mean scores for health*

Demographic parameter	MMT Group	HAU Group	t	df	p-value
(TOP)					
Physical Health Score	10.70	8.83	1.55	58	0.126
Psychological Health Score	9.57	9.27	0.22	58	0.82
Quality Life Score	5.154	4.232	0.68	58	0.49

Hazardous Alcohol Use behaviour

The mean AUDIT score for the HAU group was 24 ± 8.4 (range 9 - 38), while the mean number of units of alcohol consumed per day was 14.2 ±11.8 units (range 3-60 units). The majority of participants in this group consumed strong beer (73.3%, n = 22) compared with (16.7%, n = 5) in the MMT group, only two participants consumed wine (6.7%) and one participant reported consuming mixed kinds of alcohol (3.3%). The most common alcohol percentage consumed was 9% (60%, n = 18).

The mean MMT AUDIT score mean was 1.6 ±1.8 (range 0 - 6) as two thirds. More than half of the participants in the MMT did not consume alcohol (66.7%, n = 20). However, six participants (20%) had consumed alcohol in the past 24 hours and four (13.3%) had consumed alcohol at least once in the past week. The mean number of units of alcohol consumed per day was 1.1 ± 2.1 units (range 0 - 9 units). When alcohol was reported by the HAU group to be consumed, strong beer was the most common (16.7%, n = 5); few reported consuming spirits (6.7%, n = 2), only one participant reported drinking wine, and one reported drinking beer.

Table 4-19 *Summary baseline characteristics for MMT and HAU groups*

Characteristic	MMT	HAU	t	df	p-value
Cigarette use /day	12.50	14.62	-1.2	58	0.3
Alcohol units/day	3.71	14.18	-7.7	58	0.02

Table 4-19's results indicate that a significant difference using an indepednet t-test in parameters related units of alcohol reported. Cigarettes use was not found to be different between the two groups.

Methadone Treatment and illicit drug use

The mean methadone dose was found to be higher in the HAU group (65.6 ± 26mg; range: 30mg -130mg methadone/day) when compared to the population of the MMT group (58.4 ± 27.6 mg methadone/day; range: 25mg - 120mg methadone/day).

Table 4-20 *A summary of the methadone treatment parameters comparing MMT and HAU groups*

Demographic	MMT Group	HAU Group	t	df	p-value
Methadone dose (mean ±SD)	58.47 ±27.56	65.67 ±26.02	-1.04	58	0.30
SOWS score (Mean ±SD)	3.63 ±6.02	7.5 ±7.05	-2.2	58	0.02

Demographic	MMT Group	HAU Group	Chi-square	df	p-value
Missed dose (No. of participants)	10	17			
In the last week					
In the last month	3	6	6.66	3	0.84
Never	15	6			
In the last year	1	1			
Methadone use on top (yes/no)	8/22	9/21	0.08	1	0.77
Other medication (yes/no)	21/9	17/13	1.15	1	0.284
SOWS score (Mean ±SD)	3.63 ±6.02	7.5 ±7.05	-2.2	58	0.02
Illicit heroin use (%)	21	22	0.08	1	0.77
Crack cocaine (%)	18	23	1.93	1	0.16
Illicit benzodiazepines (%)	3	6	1.18	1	0.27
Intravenous use (%)	10	15	1.71	1	0.19

Indepednet t-test (t = -1.04, df = 58) indicated no significant difference (p = 0.30) between the prescribed doses between the methadone maintenance and the hazardous alcohol drinking behaviour group (See Table 4-20). There was no significant difference reported in the amount of the illicit drug use including heroin or crack cocaine. There was a slight difference in the number of participants who reported using illicit benzodiazepine, but it was not found to be signficiant. The SOWS score was significantly different between the two groups, indicating that patients from the

HAU were presenting with more severe withdrwal symptoms compared to the MMT group. The following section will explore scores in each item of the SOWS.

4.1.4.9 Severity of withdrawal symptoms

The independent t-test was conducted to compare the severity of withdrawal symptoms between MMT group and HAU. The severity of withdrawal symptoms was obtained using the SOWS instrument to assess withdrawal symptoms during methadone treatment. The SOWS items include feeling sick, stomach cramps, muscle spasm or twitching, feeling of coldness, heart pounding, muscle tension, aches and pains, yawning, runny eyes and insomnia. Higher score in the items indicate higher severity of withdrawal symptoms.

The mean score of withdrawal symptoms as reported from the SOWS questionnaire for the whole sample was 5.57 ± 6.79 (range 0 - 27). For the HAU group it was 7.5 ± 7.05 and for the MMT group the mean SOWS score 3.63 ± 6.02.

Table 4-21 *A breakdown of the different symptoms of opiate withdrawal for the HAU and MMT groups*

	Group	Mean	*SD	t	df	p-value
Feeling sick	MMT	0.20	0.61	-2.99	58	0.001
	HAU	0.83	0.99			
Stomach cramps	MMT	0.33	0.76	-1.03	58	0.30
	HAU	0.57	0.97			
Muscle spasm	MMT	0.17	0.65	-1.52	58	0.13
	HAU	0.47	0.86			
Feeling of	MMT	0.47	0.90	-1.53	58	0.13
coldness	HAU	0.83	0.95			
Heart pounding	MMT	0.27	0.78	-1.09	58	0.27
	HAU	0.50	0.86			
Muscle tension	MMT	0.30	0.75	-1.65	58	0.10
	HAU	0.67	0.96			
Aches and pains	MMT	0.43	0.90	-1.61	58	0.11
	HAU	0.83	1.02			
Yawning	MMT	0.37	0.72	-2.04	58	0.04
	HAU	0.83	1.02			
Runny eyes	MMT	0.27	0.69	-2.13	58	0.03
	HAU	0.73	0.98			
Insomnia	MMT	0.57	0.86	-3.27	58	0.001
	HAU	1.50	1.31			

*Standard deviation

Descriptive statistics of the ten opiate withdrawal symptoms between the two groups of patients in the MMT group and patients in the HAU group were investigated in Table 4-21. It can be observed that the patients in the HAU group have higher scores than those of the MMT group in all symptom areas: feeling sick, stomach cramps, muscle spasm or twitching, feeling of coldness, heart pounding, muscle tension, aches and pains, yawning, runny eyes, and insomnia. Furthermore, the mean scores of all

ten withdrawal symptoms were higher in those of the patients in the HAU group. The mean difference will be further validated by the t-test of difference to see if the difference is significance or not based on the t statistics at the level of significance of 0.05.

Table 4-22 *Descriptive Statistics of SOWS Scores by Grouping of Methadone Maintenance Group (MMT) and Hazardous Alcohol Use Behavior Group (HAU)*

	Group	Mean	SD	t	df	p-value
Feeling sick	MMT	0.20	0.61	-2.99	58	0.001
	HAU	0.83	0.99			
Stomach cramps	MMT	0.33	0.76	-1.03	58	0.30
	HAU	0.57	0.97			
Muscle spasm	MMT	0.17	0.65	-1.52	58	0.13
	HAU	0.47	0.86			
Feeling of	MMT	0.47	0.90	-1.53	58	0.13
coldness	HAU	0.83	0.95			
Heart pounding	MMT	0.27	0.78	-1.09	58	0.27
	HAU	0.50	0.86			
Muscle tension	MMT	0.30	0.75	-1.65	58	0.10
	HAU	0.67	0.96			
Aches and pains	MMT	0.43	0.90	-1.61	58	0.11
	HAU	0.83	1.02			
Yawning	MMT	0.37	0.72	-2.04	58	0.04
	HAU	0.83	1.02			
Runny eyes	MMT	0.27	0.69	-2.13	58	0.03
	HAU	0.73	0.98			
Insomnia	MMT	0.57	0.86	-3.27	58	0.001
	HAU	1.50	1.31			

*Standard deviation

Results of the independent sample t-test showed that the severity of the withdrawal symptoms in SOWS of feeling sick (t = -2.99, df = 58, $p < 0.001$), yawning (t = -2.05, df = 58, $p = 0.05$), runny eyes (t = -2.13, df = 58, $p = 0.04$), and insomnia (t = 3.27, df = 58, $p < 0.001$) were significantly different between patients in the methadone maintenance group and patients in the hazardous alcohol drinking behaviour group.

Urine concentration range values

In the HAU group, the mean methadone concentration in urine at trough was μg/L 2634.5 ± 3431.9 μg/L (8.38-15901.6). The mean EDDP concentration at trough was 11809.7 ±36647.8 μg/L (186.2 - 302940.2). In the MMT group, the mean methadone concentration in urine at trough was 4450.3 ± 5356.9 μg/L (11 - 20351.8) and the mean EDDP concentration at trough was 19072.5 μg/L ± 36647.8 (68 - 258161.8). The methadone concentration was divided by EDDP concentration to produce urine methadone/EDDP ratio. The mean methadone/EDDP ratio was 0.49 ± 0.62 (0.00 - 4.01) compared to the mean methadone:EDDP ratio in the HAU group which was 0.54 ± 0.56 (0.0 - 2.38).

Results of the independent sample t-test showed that the quality of life scores ($t = 0.23$, $df = 53$, $p = 0.82$), methadone concentration ($t = 1.41$, $df = 53$, $p = 0.16$), EDDP concentration ($t = 1.78$, $df = 53$, $p = 0.08$), and ratio of methadone and EDDP ($t = -1.22$, $df = 53$, $p = 0.23$) were not significantly different between the groups. Therefore, the mean differences observed in the descriptive statistics analysis were not validated.

4.1.4.10 Comparison of Severity of Methadone Dosage, Methadone Concentration, EDDP Concentration, Ratio of Methadone/EDDP, pH Levels across each Time Period

The ANOVA test was conducted to determine which of the study variables (methadone dosage, methadone concentration, EDDP concentration, ratio of methadone:EDDP, AUDIT score, SOWS score, and pH levels) were significantly different in the time period of the baseline period time 1, time 2, time 3, and time 4.

The results showed that the dependent variables of methadone dose (F = 0.26, df = 3.31, p = 0.85), methadone concentration (F = 0.42, df = 3.13, p = 0.74), EDDP concentration (F = 1.02, df = 3.13, p = 0.38), ratio of methadone:EDDP (F = 0.42, df = 3.13, p = 0.74), pH levels (F = 0.72, df = 3.13, p = 0.54), AUDIT score (F = 0.00, df = 3.13, p = 1.00), and SOWS score (F = 1.57, df = 3.13, p = 0.20) were not significantly different across the four time periods.

The urinary methadone concentration was divided by the urinary EDDP concentration to produce the urinary methadone/EDDP ratio. The mean methadone/EDDP ratio for all participants was 0.462 ± 0.36 (range 0.0 - 1.8) as shown in Figure 4-10 averaged for each week of the whole month long study for the whole group.

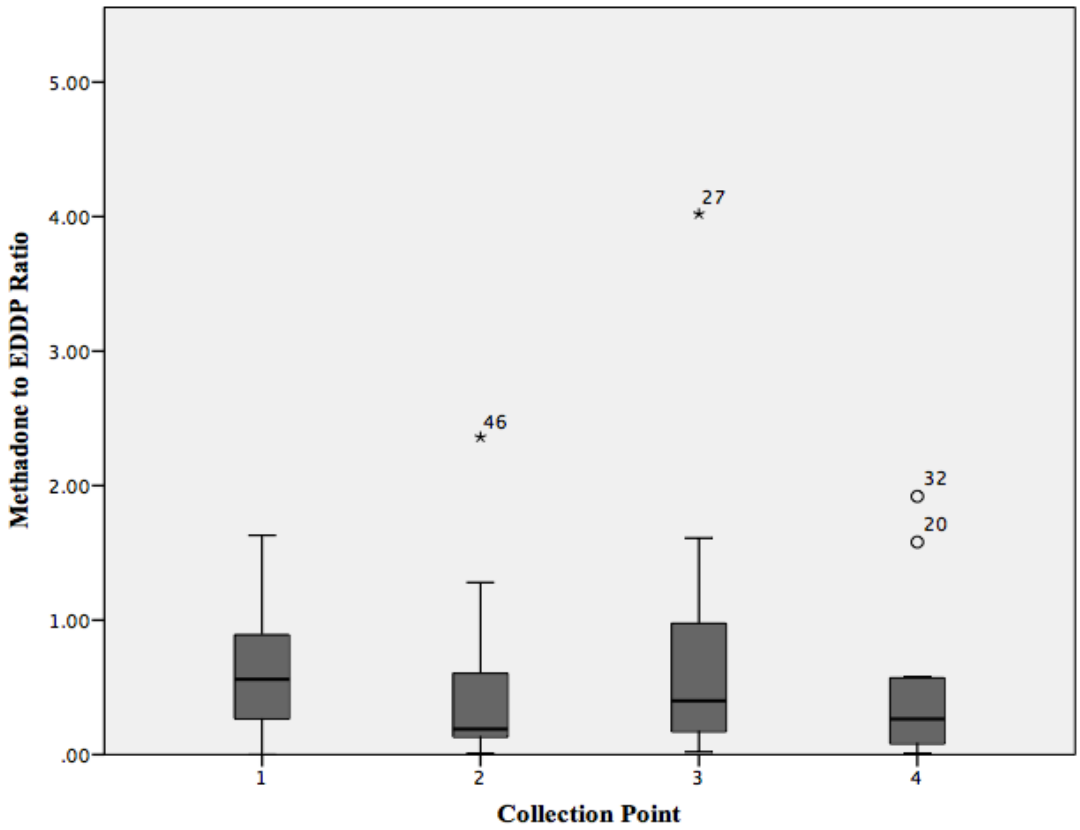


Figure 4-10 *Urinary methadone/EDDP ratio during each week of the month long study for the whole group.*

4.2 Study (1.b) Urinary methadone and EDDP measurements in patients being inducted onto methadone

4.2.1 Background

The induction phase of methadone treatment harbours a significant risk of overdose. Compared to other medications, methadone has a narrow index between the ratio of fatal dose and the maximum recommended initial dose (Repchinsky et al., 2003). Some studies reported deaths associated with low methadone doses between 30 mg and 50 mg (Nilsson et al., 1982).

An Australian study reported similar findings, with previous episodes of treatment associated with reduced risk during treatment induction (Degenhardt et al., 2009) Degenhardt and colleagues also found that the majority of these induction deaths occured in the first two treatment episodes.

Induction of CYP3A4 at the beginning of MMT probably explains, at least in part, the increased EDDP/methadone ratio, the increased clearance, the decreased elimination half-life and the decreased methadone steady-state plasma concentrations measured in patients at the first month of treatment (Wolff et al., 2000) and justifies the need for dosage adaptation.

4.2.2 Methods

Using a case series study, methadone and EDDP were measured at the onset of treatment (induction) in order to assess whether the ratio of EDDP:methadone could be used effectively as a biomarker for methadone compliance. The setting, inclusion, and exclusion criteria have been described in Chapter 5 above unless stated otherwise.

Six potential participants were approached to participate in the study, and three gave their consent.

4.2.3 Results

Three participants were recruited on the day they started their methadone. Participant number one was a 43-year-old male, who was self-referred and had no formal qualifications. He reported his ethnic background as 'Other'. His methadone-starting dose was 50 mg/day. Participant number two was a 45-year-old female who reported not having any formal qualifications and described her ethnic background as White British. Participant number three was a 55-year-old male, who was also self-referred and reported having a GCSE/O-Level qualification. He described his ethnic background as White British. All of the participants in this study were unemployed and all smoked cigarettes, with an average of 15 ± 8.6 cigarettes per day (10 - 25). None reported regular alcohol consumption and all scored zero on the AUDIT. However participant one described himself as a social drinker.

Participant one reported receiving 50 mg/day of methadone compared to participant two and three who revealed receiving 30 mg/day of methadone at induction. When asked about receiving any other medication, only participant one reported receiving medication – he was prescribed an antidepressant.

Participants also reported a history of using illicit methadone, with participant number one reporting using up to 200 mg of illicit methadone compared to participant two who reported using only 30 mg, and participant three who reported using 20 mg.

Participants were asked about their illicit drug use in the past 28 days. Table 4-23 summarises the illicit drug use reported. All participants reported using heroin with

participant number three reporting the highest amount of heroin use in the past month. Only participant one reported use of other illicit drugs: crack cocaine, cannabis and benzodiazepine which he described as a 5 mg valium pill.

Table 4-23 *Illicit drug use within methadone induction group as reported using the Treatment Outcome Profile (TOP)*

Participant	Opiate	Amount (g per month)	Crack Cocaine	Cannabis	Amount (Roles per month)	Benzodiazepines	Amount (mg per month)
1	Yes	4.2	Yes	Yes	80	Yes	140
2	Yes	5.6	No	No	-	No	-
3	Yes	16.8	No	No	-	No	-

4.2.3.1 Biological measurements

A daily sample of urine was collected every day for 13 days from each participant. Table 4-24 summarises data from participant number one. The mean methadone concentration was 1467.2 ± 1595.9 (58.9 – 6506.8), the mean concentration of EDDP was 4151.4 ± 2819.7 (1108.7 – 107080.1), and the mean concentration of the EDDP:methadone ration was 4.6 ± 4.6 (2.3 – 18).

Table 4-24 *Daily methadone urine concentration, EDDP urine concentration, and ratio of EDDP/methadone for participant 1*

Patient	Day	Dose (mg)	Methadone Concentration (ug/L)	EDDP concentration (ug/L)	pH	EDDP:methadone ratio
1	1	30	788.4	3891.8	7	1.2
	2	30	58.9	1108.7	8	1.5
	3	30	1218.9	1844.7	6	1.6
	4	40	1776.9	2231.3	6	1.6
	5	40	638.6	1793.9	6	1.7
	6[a]	50	589.5	1667.8	7	2.1
	7[a]	50	1065.3	2526.2	6	1.7
	8	50	2052.9	4153.8	6	1.9
	9	50	1100.5	7408.1	7	1.6
	10	50	897.5	6789.1	7	1.0
	11	50	1163.1	5136.8	7	1.4
	12	60	6505.8	10780.1	6	2.7
	13[a]	60	1217.8	4636.0	7	2.5

[a]Weekend

Table 4-25 summarises data from participant number two. The mean methadone concentration was 2626.1 ± 3694.4 (40.7 – 9843.5), the mean concentration of EDDP was 5855 ± 7080.8 (215.2 – 23143.5) and the mean concentration of the EDDP:methadone ration was 6.6 ± 11.9 (1.5 – 42.4).

Table 4-25 *Daily methadone urine concentration, EDDP urine concentration, and ratio of EDDP/methadone for participant 2*

Participant	Day	Dose (mg)	Methadone Concentration (ug/L)	EDDP concentration (ug/L)	pH	Ratio
2	1	30	778.7	2216.4	7	2.8
	2	40	1246.1	5725.2	7	4.6
	3*	-	-	-	-	-
	4	0	161.6	350.7	7	2.2
	5	40	160.7	1045.0	8	6.5
	6*	-	-	-	-	-
	7	0	-	53.1	7	-
	8	40	9797.2	23143.5	6	2.4
	9*	-	-	-	-	-
	10	0	40.7	1724.3	9	42.4
	11	40	2626.6	6469.7	6	2.5
	12	40	9843.5	14536.8	6	1.5
	13	40	2988.6	6418.3	6	2.1
	14[a]	40	1189.5	2560.1	6	2.2

*Missed methadone dose the day before

[a]Weekend

Table 4-26 summarises data from participant number three. The mean methadone concentration was 2481.9 ± 1461.8 (245.7 - 5461.4), the mean concentration of EDDP was 4021.2 ± 2431.6 (614.1 - 8958.9) and the mean concentration of the EDDP:methadone ration was 1.7 ± 0.5 (1 - 2.7).

Table 4-26 *Daily methadone urine concentration, EDDP urine concentration, and ratio of EDDP/methadone for participant 3*

Participant	Day	Dose (mg)	Methadone Concentration (ug/L)	EDDP concentration (ug/L)	pH	EDDP/methadone Ratio
	1	50	4033.8	4823.1	6	1.2
	2	50	2626.8	3918.4	6	1.5
	3[a]	40	5461.4	8958.9	6	1.6
	4[a]	40	3307.8	5317.6	6	1.6
	5	40	3250.7	5559.1	6	1.7
	6	50	2906.3	6206.8	6	2.1
3	7	40	2975.7	5111.2	6	1.7
	8	50	2551.9	4973.6	6	1.9
	9	50	1690.6	2649.8	6	1.6
	10[a]	50	1672.0	1615.7	6	1.0
	11[a]	50	1258.4	1769.2	6	1.4
	12	50	283.7	757.6	7	2.7
	13	50	245.7	614.1	7	2.5

[a]Weekend

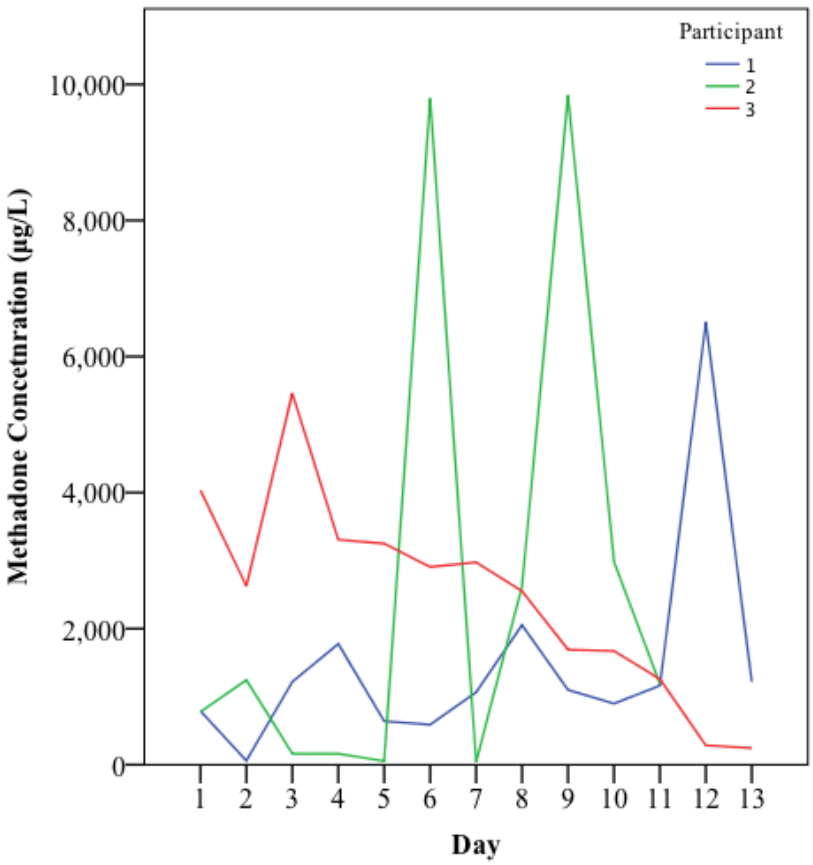


Figure 4-11 *Presentation of three participants methadone concentration across the days of collection*

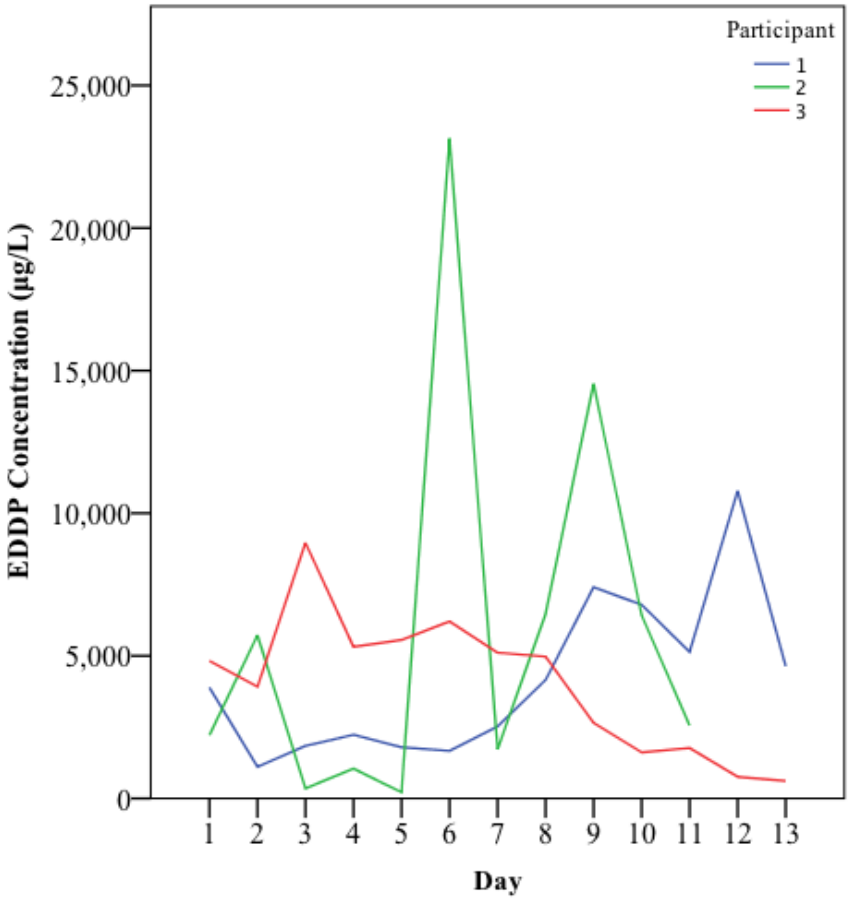


Figure 4-12 *Presentation of three participants' EDDP concentration across days of sample collection*

A One-way ANVOA test indicated that there was no significant difference between the means of the methadone concentrations ($F = 0.82$, $df = 1$, $p = 0.43$) or mean of EDDP concentrations ($F = 0.7$, $df = 1$, $p = 0.5$). However, the EDDP:methadone ratio presented a significant difference ($F = 3.1$, $df = 1$, $p = 0.05$) between the three patients.

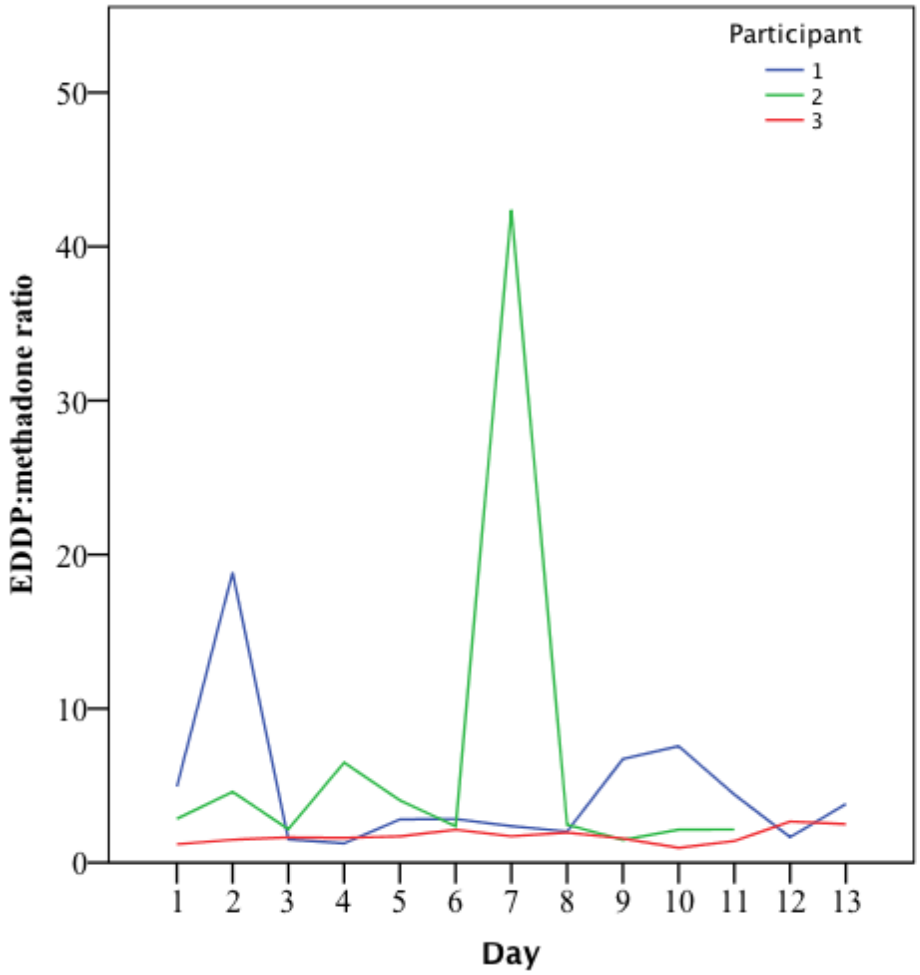


Figure 4-13 *Presentation of three participants' EDDP concentration across days of sample collection*

From figure 4-1 we can see that patient number two had a peak of EDDP and methadone ratio. The other patients, however, exhibited ratios that were more stable, especially patient number three. The ratio seems to slightly increase on day number 13 compared to day 1. Patient one reported the highest dose (50 mg); however, his ratio peaked on the second day of induction and then stabilised, with a slight peak on the 9th and 10th days.

4.2.4 Discussion

Methadone was first introduced as a substitution treatment for heroin dependence in the 1960's. The effectiveness of methadone was reflected in the reduction of crime, of the risk of HIV and Hepatitis C infections, and of mortality. The stabilisation and retention in the treatment led to patients increasing their contribution to society (Farrell et al., 1994).

As outlined in Chapter 1, previous studies have focussed on plasma concentration of methadone; the relationship between dose and its trough plasma concentration has been established (Rostami-Hodjegan et al., 2001). However, using plasma in a clinical setting as a means to assess patients' compliance is not routinely performed. Methadone concentration has also been investigated in urine samples. Recent studies have investigated methadone, its inactive metabolite EDDP, and the attempts to utilise these concentrations as a mean of monitoring patients' compliance. However, a high degree of inter-individual variability has been reported. This may be due to the nature of the design of the studies in which samples were not linked to the clinical participants' history including use on top of prescribed doses.

The first study investigated the trough urinary concentrations of methadone and EDDP in sixty patients receiving methadone treatment to address the potential of utilisation of EDDP:methadone ratio as a biological tool to assess patients' compliance.

The main finding indicated that the over the 4 points of collection there was no significant difference in the EDDP:methadone ratio. However, it was reported with high inter-individual variability. Further variables that were examined, including methadone concentration, EDDP concentration, SOWS scores, EDDP:methadone

ratio and methadone:EDDP ratio, did not present significant differences. This may be due to the fact that the patients collected in this study were all maintained on methadone during a steady state.

Further investigation of the samples in the study reported extremely high values of the EDDP:methadone ratio compared to previous studies. Kreek et al. (1973) reported a mean ratio of 1.55 and range of 0.45-5.07 in urine samples collected from participants receiving a methadone dose between 80 mg and 100 mg. The high variability in this study's results may be partially due to the fact that samples were collected at trough rather than 24-hour urine collection. However, it is more clinically realistic to collect at trough, as patients will be visiting the clinic to collect their daily dose and that is the most suitable time to collect urine samples to assess compliance.

The study also found that the concentration of EDDP was higher than methadone in urine samples. The mean urinary methadone concentration at trough for the whole study population was 3497.23 ± 4553.76 µg/L (5.90 - 20351.8) and the mean urinary EDDP concentration at trough was 15106.9482 ± 36208.33 µg/L (0.05 - 302940). The concentration presents a high variability, which is consistent with previous findings indicating that the high variability measured in urinary concentrations limited the possible utilisation of excretion data (George & Braithwaite, 1999). However, unlike previous studies, our study investigated the concentrations of methadone and EDDP and the ratio between them at specific doses, and samples were collected at trough to control time of consumption variability.

To further control variability, the samples in the study were analysed according to the doses that each participant reported receiving. Although the variability was still detected, the mean ratio was 17.8 ± 37.48 (0.5 – 142.6) in participants who received a

dose of 40 mg/day compared to 0.9 ± 7.5 (1.1 – 31.3) in urine samples in which participants reported receiving 50 mg/day. This variation between the ratios may be due to outliers in the group, who, when investigated, included a participant that had only been on methadone for 10 days, which might account for the variability in ratio.

A study by Nielsen et al. (2013) investigated the possibility of utilising the ratio as a means of predicting the cause of death in patients receiving methadone maintenance. They predicted that it would be in those patients who had abstained from treatment, rather those who had complied, in which a wide difference between the metabolite and drug would be observed. The observation of this phenomenon was apparent in a few cases; however, only one participant was reported to have died. The cause of death was not linked to the methadone dose; instead it was due to alcohol complications.

Methadone concentration and EDDP concentrations were also investigated separately. In accordance with previous findings, methadone and EDDP concentrations were significantly correlated with the methadone dose administered. However, this positive relationship was observed to become slower as the dose increases. This could be explained by the non-proportional change of metabolic formation when the metabolic pathway is saturated.

The most common methadone dose of the 138 samples collected in the study was 40 mg, closely followed by 50 mg. This indicated that the patients in this study were prescribed a lower dose than the 80 mg that other studies have recommended as an effective dose (Dole et al., 1965). High doses of methadone prescription have been found to be associated with higher treatment retention and less illicit drug use (Farrell et al., 1994). This is consistent with the findings of the results of self-report related to

illicit drug use in this study, with a high prevalence of heroin and cocaine use, where more than half of the participants (72.7%, n = 43) self-reported that they used heroin and also crack cocaine (68.3%, n = 41). Other substances were used less frequently; however, some participants reported using cannabis and benzodiazepine. These results indicated a strong correlation between dose, methadone concentration, and EDDP concentration. This is consistent with results presenting the relationship at trough between methadone dose and plasma (Rostami-Hodjegan et al., 2001); however, the study found a high degree of inter-individual variability, in which dose explains only a small part of the variation in methadone plasma concentration (Eap et al, 2000).

Comparison between methadone maintenance and hazardous alcohol users

In general, the observed findings show that the opioid co-dependent participants (HAU) had significantly higher scores on the withdrawal symptoms scales when compared with methadone maintenance group (MMT), who were identified by scroing less than 8 on the AUDIT score. These results indicate that patients who present with problematic alcohol use during the methadone maintenance can exacerbate withdrawal symptoms.

This significant difference in the SOWS score between MMT and HAU groups could support a link between the endogenous opioid system and excessive alcohol consumption. Withdrawing from either of these types of substances affects the noradrenalin, GBA and NMDA neural circuit as well the depletion of dopamine levels (Volkow et al., 1999; Gianoulakis, 2001; de Wet et al., 2004).

The literature indicates that alcohol consumption in moderation stimulates the release of opioid peptides in those brain regions that are associated with reward and reinforcement, and mediates the reinforcing effects of alcohol. However, it was found that the central opioid deficiency is induced when larger amounts alcohol are consumed. This may be the reason why patients percieve the symptoms as opioid withdrawal, which may promote alcohol consumption through the mechanisms of negative reinforcement (Gianoulakis, 2001).

These findings were in parallel with the concentration levels observed in which the methadone concentration was significantly lower in the HAU group than in concentrations measured in the MMT group. This could be a reflection of the fact that methadone metabolism is directly affected by alcohol consumption. This is in line with previous studies that show that alcohol consumption can increase methadone peak concentration when it is acute but cab decrease methadone concentration when it is chronic (Clark et al., 2006). This can also be correlated with the fact that patients from the hazardous alcohol use group scored significantly higher on their SOWS scores than those in the MMT group. The more severe the withdrawal symptoms, the more likely patients are to use heroin on top and not comply with their methadone maintenance. The EDDP:methadone ratio was also significantly different between the two groups, which could again be related to methadone metabolism.

Methadone and EDDP ratio in urine

Previous studies have addressed the urinary excretion of methadone and its inactive metabolite EDDP (Leimanis et al., 2012), but few studies have addressed the potential of using methadone/EDDP ratio as a marker for metabolism in methadone treatment

by investigating the ratio at different stages of treatment and investigating the effect of alcohol consumption on the methadone/EDDP ratio.

Although both EDDP and methadone concentrations indicated a positive relationship between both methadone concentration and EDDP concentration in urine and the methadone dose, EDDP was always reported to be higher concentration than methadone. However, the results also indicate variability in the methadone and EDDP concentrations between the participants in the group analyzed (HAU) but remained constant within individual participants over the period of a month. This is consistent with other research that has investigated methadone and EDDP concentration. However, the patients in these studies were receiving methadone for pain management, indicating much lower levels of methadone and EDDP concentrations (Leimanis et al., 2012).

Induction is a critical stage of methadone treatment where the main dose adjustment occurs (Krambeer et al., 2001). It was indicated that there was no significant relationship between the EDDP:Methadone among the three patients. Previous studies managed to identify a 6-fold increase in the ratio during induction period; however, the study results indicated an increase in the ratio in only one patient. Patient number two exhibited a strikingly different pattern characterised with a peak during the days the patients missed her doses. The patient revealed in her self-report questionnaire a history of using illicit methadone which could be argued as responsible for the spikes in the graphs. Further collection of urine samples for up to 40 days might have been useful. A limitation of this study was that the patients were asked to collect and store their urine samples over the weekend. Future work investigated patients in an

inpatient setting might help with eliminating variables that can affect the EDDP:methadone ratio.

Demographics and characterisation

The majority of patients in the study were male (62%). This correlates well with previous findings, indicating higher numbers of male patients receiving treatment for opioid dependence (Marsden et al., 2000; Gerra et al., 2003; Senbanjo et al., 2007). However, it is important to highlight that the smaller number of female patients does not indicate that the problem is less severe in this group.

More than half of the sample (57%) described themselves as White British, although this could be a reflection of the location of the study in the South-East London borough of Southwark. In this borough, the local authority's statistics suggest that White British is the common ethnicity (Gossop et al., 2003).

The study sample were socially deprived; all of the participants reported that they were unemployed, and the majority had secured no formal educational or vocational qualifications. The influence of social deprivation has been argued to affect the level of alcohol consumption in general, as well as that among patients receiving methadone maintenance treatment (Rodham et al., 2005; Senbanjo et al., 2007).

Drug use and injecting behaviour

Throughout the interviews, illicit drug use was high in both groups during the maintenance phase. The pattern of use was also very similar, with no amphetamine and only one case of powdered cocaine reported. However, heroin use continued

throughout the treatment, indicating non-compliance. There was no significant difference between the reported drug use between the groups MMT and HAU groups.

Limitations

The potential limitations of the study include the following:

Recruitment and sampling method

The present study requited *participants at 4 points* ideally once a week. This was to investigate variability in the ratio over time within the same participant; however, due to the sometimes chaotic nature of the participants in the study it was difficult to ensure compliance with itnerviews.

Further sample collection was more successful with participants from the alcohol hazardous group because they were required to collect their methadone dose from the outpatient centre and to use a breathalyser before receiving their daily dose. However, it was sometimes difficult to engage with the participants when they presented with alcohol withdrawal symptoms. The urine sample was required to be collected before the participants received their daily dose (at trough), which was problematic with patients who exhibiting exhibited methadone withdrawal symptoms.

4.2.4.1 Conclusion

Concentrations of methadone and EDDP exhibited high variability in EDDP:methadone ratio. Although attempts to control some variables were presented in some sub-experiements in the study including investigating gender difference, dose, and alcohol consumption, variations were still observed. Although EDDP:methadone ratio might not be a specific tool for compliance, measuring the

EDDP and methadone can be utilised as specific biomarkers to indicate that patients had consumed their dose.

4.3 Recent Alcohol Consumption Biomarkers (EtG and EtS) in Patients Receiving Methadone Substitution Maintenance Treatment

4.3.1 Background

Alcohol use among methadone maintenance patients has been well documented in literature. However, a means of assessing alcohol use in this population using objective measures has not been well established. Urine screening for illicit drug use is routinely clinically applied. Alcohol use among patients receiving methadone, although problematic, is not necessarily dependence. However, in some cases it may affect treatment outcome and can even lead to death. Traditional biomarkers measure alcohol indirectly and are therefore limited in aspects related to the time period since last consumption. Recently, there has been a focus in measuring direct alcohol metabolites including EtG and EtS. Data on the utilisation of the direct biomarkers of alcohol consumption (EtG and EtS) are limited. For this reason, using a cross-sectional prospective cohort design, Study 2 investigated the effectiveness of Ethyl glucoronide (EtG) and Ethyl Sulphate (EtS) to screen for recent alcohol consumption in patients collecting their daily methadone dose. The purpose of the study was to help establish whether these new biomarkers could be used as tools for indicating alcohol use among clients receiving methadone substitution maintenance treatment.

4.3.2 Methods

Sixty participants were recruited and provided signed written informed consent. Self-reported data were collected regarding licit and illicit drug use, methadone treatment and other current treatment (See Appendices E and F). A urine sample (20 mL) voided unobserved was collected after the interview, and before administration of the daily dose of methadone (at trough). The study was conducted over a period of one month and participants were asked to provide a urine sample each week. The urine samples were frozen in the laboratory at -20° C until analysis. The consecutive interviews included a shorter version of the self-report questionnaire and related to alcohol and drug use in the past 24 hours. Urine samples were analysed for recent alcohol consumption biomarkers EtG and EtS using Liquid Chromatography coupled with Mass Spectrometry, as described in Chapter 5.

4.3.3 Results

Sixty participants, who were more than two weeks into methadone treatment, were recruited to the study (see chapter 6). Almost half of the participants (53.3%, n = 32 were in the age range of 39 to 47 years old. The mean age of the whole sample was 41.67 ± 8.3 years (range 23 – 56 years). The majority of the participants described themselves as White British (58.3%, n = 35) and more than half of the participants were male (65%, n = 39). The mean methadone dose at baseline for the 60 participants was 62 ± 26.8 mg methadone/day (range 25 –130 mg methadone/day).

4.3.3.1 Alcohol Use Behaviour at Baseline

Participants reported their alcohol use and results indicated that the mean number of units of alcohol consumed per day was 5.4 ± 6.2 units per day (0.0 – 40 units per day).

Participants mostly reported drinking strong beer (45%, n = 27). Some reported drinking ordinary beer (33.3%, n = 20), and a few reported drinking wine or spirits. Only one participant reported mixing the kinds of alcohol he or she drinks. The mean score of AUDIT was 13.1 ± 13.1 (0 – 38). Table 4-27 summarises the frequency of the amount of alcohol consumed per day and AUDIT scores in the cohort.

Table 4-27 *Alcohol Use By Participants and AUDIT scores*

Participant (n)	AUDIT score	Alcohol Units per day	Participant (n)	AUDIT score	Alcohol Units per day
1	2	0	31	30	19.5
2	3	9	32	26	28
3	0	0	33	26	12
4	3	3	34	27	6
5	0	0	35	38	5
6	3	0	36	29	10
7	2	0	37	9	9
8	6	0	38	15	9
9	0	0	39	33	21
10	0	0	40	17	9
11	0	0	41	24	4
12	0	0	42	31	9
13	1	0	43	31	8
14	0	0	44	28	12
15	1	0	45	37	12
16	2	0	46	18	3
17	2	0	47	36	30
18	0	0	48	12	2
19	1	3	49	12	4
20	1	0	50	30	20
21	2	0	51	16	15
22	1	4	52	28	15
23	6	3	53	33	32
24	0	0	54	22	15
25	6	0	55	30	16
26	0	0	56	26	12
27	2	3	57	20	6
28	4	1	58	9	6
29	0	0	59	32	27
30	1	0	60	16	9

Of the cohort (33.3%, n = 20) described their selves as non-drinkers. The mean number of units of alcohol per day consumed by the 40 participants was 12.4 ± 11.5 (range 1 – 60) units of alcohol. The mean score for the Alcohol Use Disorders Identification Test (AUDIT) for the drinkers in the cohort was 13.1 ± 13.1 out of a possible 40 points (range 0 – 38 points). Out of the total cohort, 11 participants (18.3%) scored zero on the AUDIT score.

Table 4-28 summarises the number of participants at risk level depending on AUDIT score. Although half of the participants were considered at low risk according to their AUDIT score (50%, n = 30), twenty-one participants (35%) were at high risk and were considered likely to be alcohol dependent.

Table 4-28 *Number of participants at risk level depending on AUDIT score*

Risk Level	AUDIT Score	No. of Participants	Percentage (%)
Low risk	0-7	30	50
Hazardous level	8-15	5	8.3
Harmful level	16-19	4	6.7
Dependence likely	20-40	21	35
Total		60	

4.3.3.2 Urinary measurements of EtG and EtS

Of the 60 participants receiving methadone maintenance, 56 urine samples were collected at baseline. Following baseline sample collection, the study attempted to collect a further three weekly urine samples from each participant. In total, 138 urine samples were collected from the 60 participants.

Table 4-29 *The provision of samples by the participants is shown*

Point of collection	Number of samples collected	Number participants
Baseline	56	22
2	35	4
3	30	13
4	18	17
Total	138	56

A synopsis of the 56 samples collected at baseline is summarised in Table 7-3). A total of 138 samples were analysed for EtG and EtS. In those who screened positive for both EtG and EtS, the mean urinary concentration was 115.11 ± 199.87 mg/L (range 0.07 – 1000 mg/L) and the mean urinary EtS concentration was 49.32 ± 82.46 mg/L (range 0.70 – 445 mg/L).

In the positive urine samples, a statistical comparison between EtS and EtG concentrations using a Spearman's Rank revealed a highly significant linear correlation across the different amounts of alcohol consumed. There was a strong relationship between EtG and EtS (r_s = 0.97, df = 136, $p<0.001$). R^2 was 0.951, indicating strong linearity, as shown in Figure 4-14.

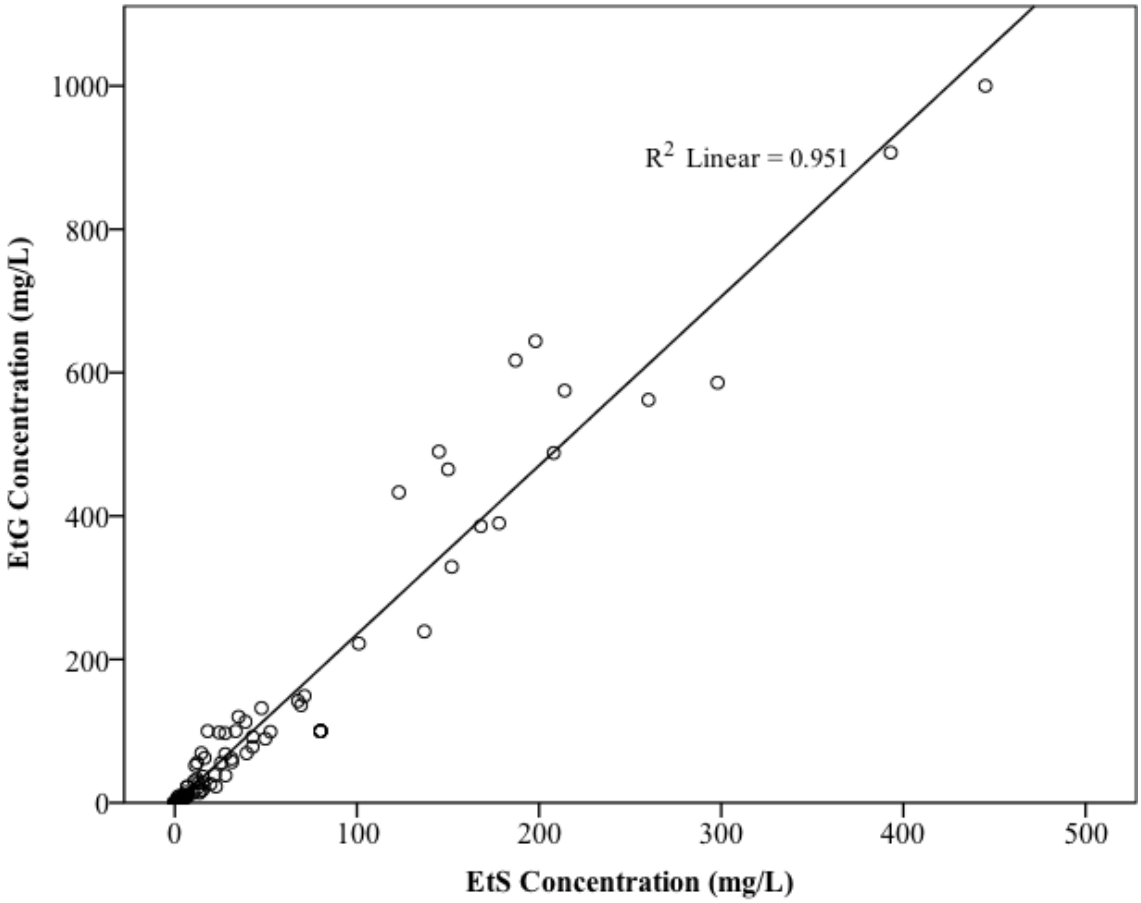


Figure 4-14 *Scatter plot of EtG concentration in relation to urinary EtS concentration (n=138) presenting a strong linearity ($p< 0.001$, $R^2=0.951$)*

The mean of the EtG/EtS ratio was 2.14 ± 1.06 (range 0.39 – 2.14) for the subjects who were drinkers.

EtG and EtS urine concentration and self-report (AUDIT score)

The mean urinary EtG and EtS concentrations were calculated in all the samples collected (n = 138) and the range was calculated according to each alcohol risk level group depending on their AUDIT score (see Table 4-30).

Table 4-30 *Descriptive statistics for urinary EtG and EtS concentration identified according to alcohol risk level in the group*

	Low risk (n=68)	**Hazardous level (n=11)**	**Harmful level (n=8)**	**Dependence likely (n=20)**
EtG Concentration Mean ± SD (Range)	7.48 ± 21.69 (0.05 -99.0)	114.61 ± 295.32 (0.05- 1000)	159.39 ± 186.18 (9.94- 586)	168.77 ± 219.32 (0.05- 907)
EtS Concentration Mean ± SD (Range)	3.50 ± 9.51 (0.02 - 52.5)	48.95 ± 131.71 (0.05 - 445)	91.38 ± 93.03 (4.58 - 298)	68.6 ± 84.53 (0.05 - 393)

Further investigation was carried out to determine if there was an association between urinary EtG concentration and AUDIT score shown in Figure 4-15. The AUDIT score can be seen in Figure 4-15. Using Pearsons Correlation coeffecient a significant linear correlation was found between urinary EtG concentration and AUDIT score ($r = 0.43$, $df = 136$, $p<0.001$). This indicates that urinary EtS concentration also correlated significantly with AUDIT score ($r = 0.42$, $df = 136$, $p<0.001$).

Results of a One-way ANOVA indicated significant differences in both EtG ($F = 10.1$, $df = 3$, $p< 0.001$) and EtS ($F = 10.9$, $df = 3$, $p< 0.001$) concentrations across risk

level groups (low risk, hazardous level, harmful level, alcohol dependent). This was followed by a Post Hoc test using Bonferroni, which indicated that the EtG concentration in the group with AUDIT scores ≥ 20 (M = 161.28, SD = 30.18, p = 0.01) was significantly higher compared with the other AUDIT score groups. The EtS concentration in the group with AUDIT scores ≥ 20 (M = 64.55, SD = 12.41, p = 0.01) was also significantly higher compared with the other AUDIT score groups.

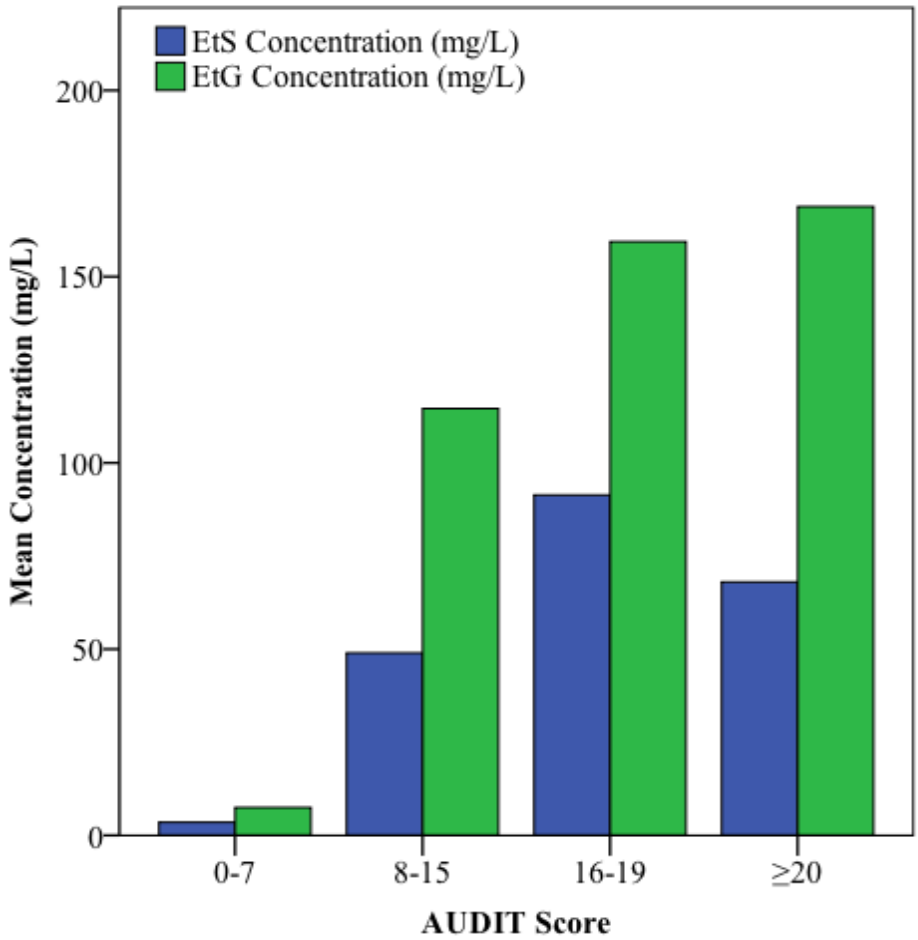


Figure 4-15 *Average urinary concentration of EtG and EtS in different risk level groups depnding on AUDIT score. One-way ANOVA analysis revealed significant differences in EtG concentrations (p < 0.001) and EtS concentrations (p < 0.001) between participants scoring (0-7) compared to participants scoring (>20)*

EtG and EtS urine concentration and self-report

At baseline, 56/60 participants had trough urine samples analysed for EtG and EtS and this was compared to self-reported alcohol use in the past week. Of the 56 participants, 33 reported consuming alcohol in the past 24 hours while seven participants reported consuming alcohol earlier in the week. Twenty participants reported not drinking (33.3%).

Related samples were analysed for EtG and EtS and found most of the samples were positive for both EtG and EtS (70%, n = 42) and only five samples were negative (8.3%). However four participants (7.1%) were only positive for EtS, which may indicate drinking earlier in the week. Five participants were only positive for EtG, which could be attributed to sample fermentation or degradation due to bacterial growth.

During the rest of the study participants were asked about their alcohol use in the past 24 hours. In the further 78 interviews that took place after the initial baseline interviews, alcohol use was reported forty-nine times (62.8%) while twenty nine (37.2%) reported not using alcohol in the past 24 hours. Urine samples were collected and analysed for EtG and EtS (n=78). Only 14 samples (17.9%) presented with negative urine EtG and EtS. Fifty-seven samples (73.1%) were positive for both EtG and EtS while 4 (5.1%) were only positive for EtG; and 3 samples (3.8%) were positive for EtS only.

EtG and EtS urine concentration and alcohol units consumed

Pearson Correlation analysis established a significant relationship between EtG and self-reported alcohol use ($r = 0.46$, $df = 136$, $p < 0.001$).

The amount of alcohol consumed in the past 24 hours reported by participants was

found to be 5.4 ± 6.2 units per day (0.0 – 40 units per day). A Spearman Correlation indicated that there was a significant correlation between the level of consumption and the EtG (r_s = 0.71, df = 136, p< 0.001) and EtS (r_s = 0.72, df = 136, p< 0.001) concentrations identified in participants' urine samples (see Figure 4-16).

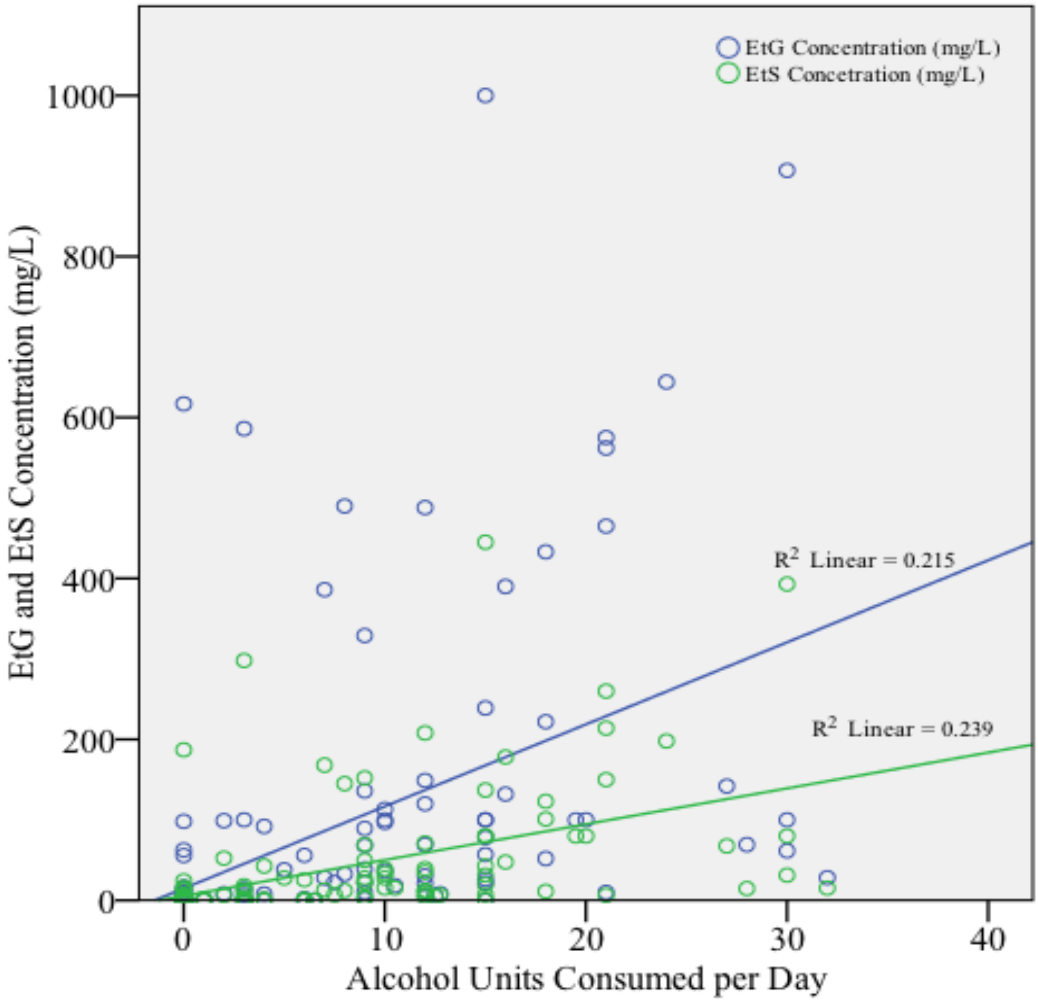


Figure 4-16 *Scatter plot of EtG and EtS urinary concentration (mg/L) in samples collected from participants receiving methadone substitution (n=138) in relation to amount of alcohol consumed per day (units)*

Comparison of male and female participants for urinary EtG and EtS

Comparison of the demographics, AUDIT score, SOWS scores, EtG, and EtS concentration between female and male participants was conducted. An independent t-test was used to compare these variables between female and male participants. A level of significance of 0.05 was used in the statistical analysis.

Table 4-31 summarised the comparison of demographic data, which include age, and AUDIT score, divided among the female and male patients as well as the p-value of the t-test to show the significance of the difference of the two gender groups. All the p-values in Table 4-31 were greater than the level of significance except for age, indicating that there were no significant differences between the genders in the study. A comparison of the opiate withdrawal scores at baseline between female and male participants was also conducted and the results showed that there no significant difference was indicated ($t = -0.27$, $df = 58$, $p = 0.77$). This indicated that the female participants did not experience more severe withdrawal symptoms compared to those of the men in the study group.

Table 4-31 *Demographics and alcohol use behaviour at baseline for male and female participants*

	Male (n=39)	**Female (n=21)**	**t**	**df**	**p-value**
Age **Mean ± SD**	43.6 ± 7.9	38 ± 7.9	-2.63	58	0.01
AUDIT score	12.9	13.6	0.19	58	0.85
Self reported alcohol use (yes)	25	15	-0.57	58	0.57
Methadone dose (mg/day) **Mean ±SD**	60 ± 26	65 ± 28	0.67	58	0.51

Table 4-32 summarises the comparison of EtG, EtS, and ratio EtG/EtS concentration divided among the female and male patients as well as the p-value of the t-test to show the significance of the difference between the two gender groups. The p-value of the t-test showed that the EtG, EtS, and ratio EtG/EtS concentration failed to show a statistical significant difference between men and women, since all the p-values were greater than the level of significance of 0.05.

Table 4-32 *The concentrations of biomarkers for male and female participants in the study*

	Male (n=39)	**Female (n=21)**	**t**	**df**	**p-value**
Mean EtG Conc. (mg/L)	89.3	69.8	-0.57	135	0.56
Mean EtS Conc. (mg/L)	36.8	33.5	-0.23	135	0.93
EtG/EtS ratio	2.12	1.82	-0.71	135	0.52

Figure 4-17 presents the mean urinary concentration of EtG and EtS in female and male participants using One-way ANOVA analysis which revealed no significant difference between them.

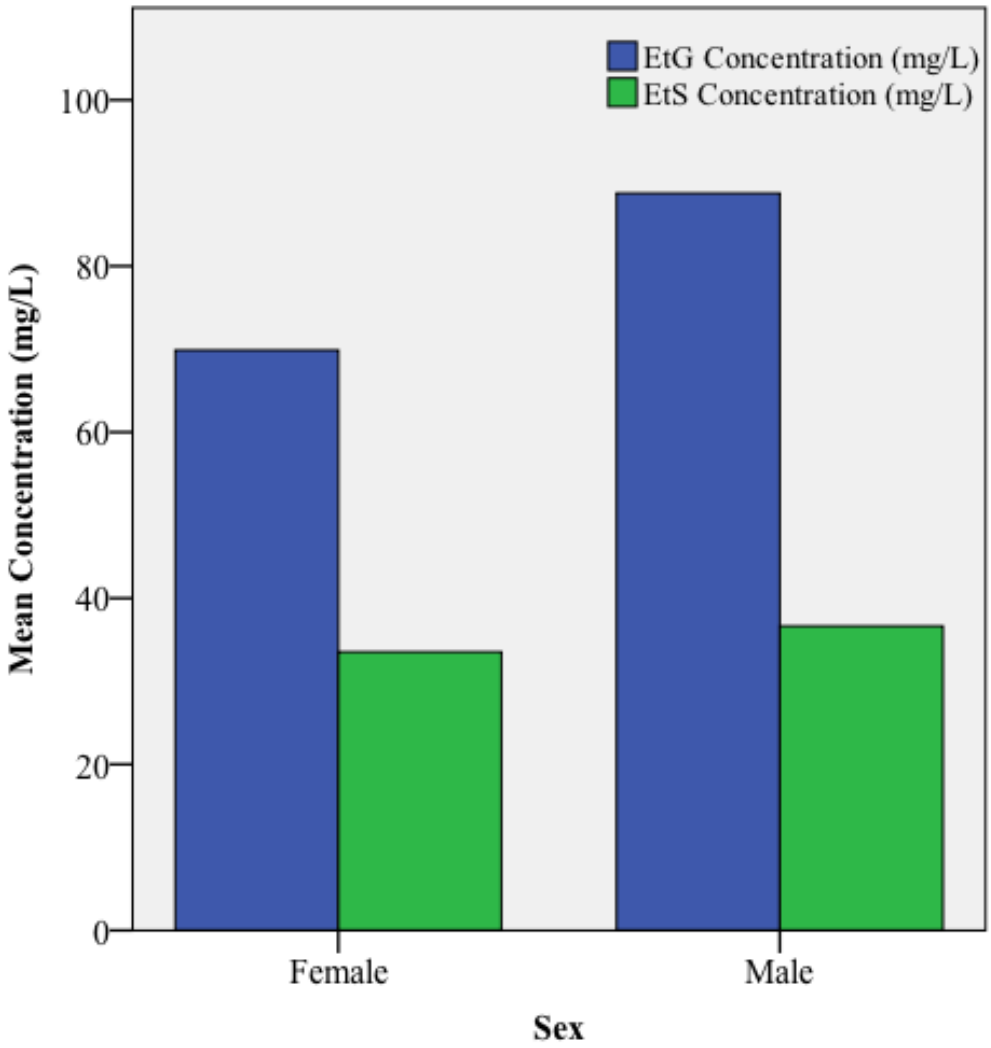


Figure 4-17 *Mean urinary concentration of EtG and EtS in female and male participants One-way ANOVA analysis revealed no significant difference between them*.

4.3.3.3 The relationship Between Methadone Concentration and Alcohol Self Report and Objective measure

Table 4-33 summarises the descriptive analysis of methadone concentration in the different risk group according to the AUDIT score. A one way ANOVA followed but a Post Hoc test found no significant difference between the groups and the methadone concentration ($F = 1.8$, $df = 3$, $p = 0.1$).

Table 4-33 *Description statistics for the methadone concentration in alcohol risk level group*

Risk Level	Methadone Concentration Mean (ug/L)	Std. Deviation	95% Confidence Interval for Mean		Min	Max
			Lower	Upper		
Low risk (0-7)	4346.44	5452.05	2925.62	5767.25	5.9	20351.8
Hazardous (8-15)	1562.77	1865.20	309.70	2815.83	108.6	5516.8
Harmful (16-19)	2325.71	2552.51	-34.96	4686.38	192.74	6506.3
Dependent >20	2944.34	3534.93	1894.59	3994.09	49.74	15901.6
Total	3458.13	4497.2	2655.40	4260.85	5.9	20351.82

Figure 4-18 indicates that there was no correlation found between EtG and methadone concentration.

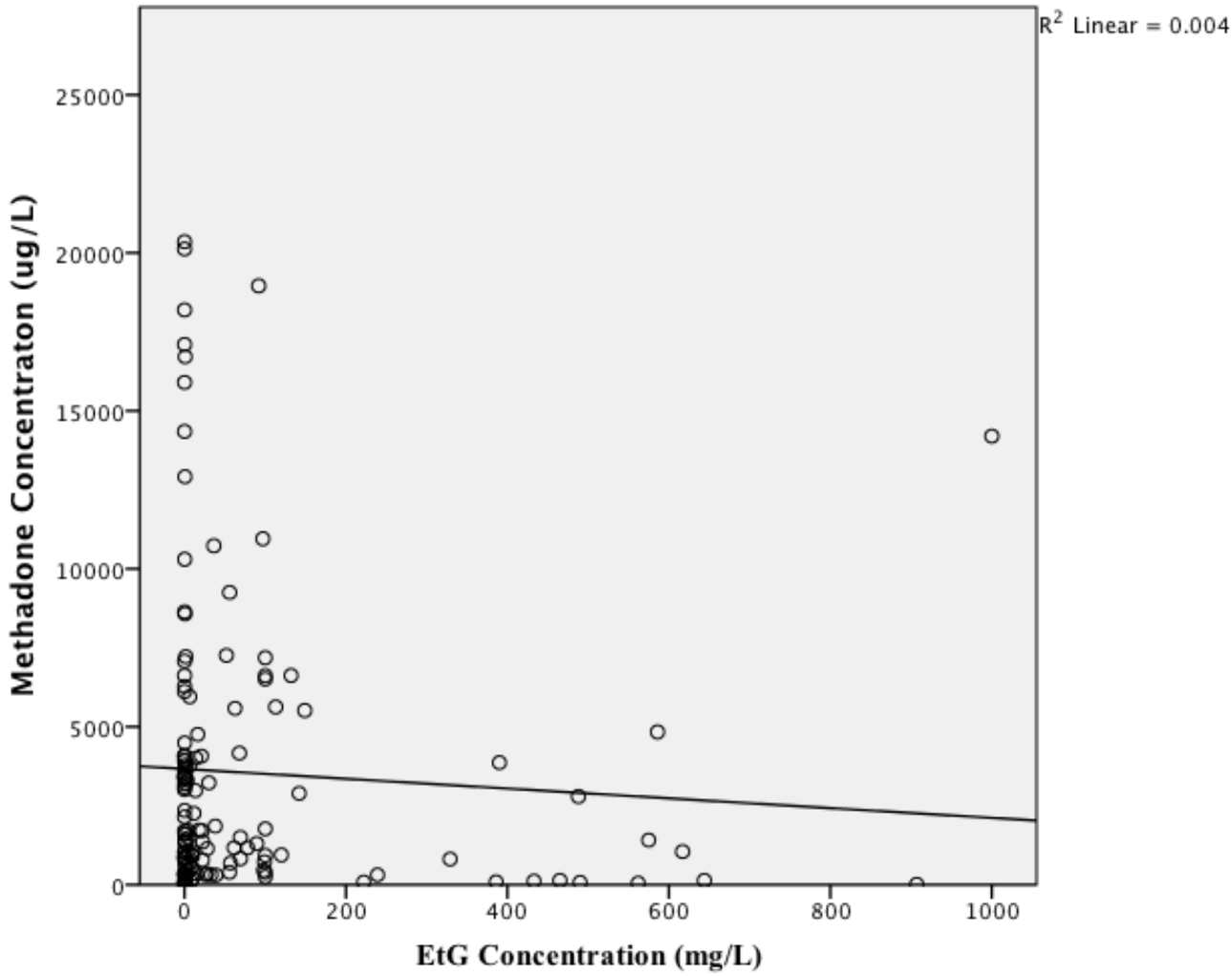


Figure 4-18 *Scatterplot presenting the relationship between EtG and methadone concentration*.

From figure 4-18 we can observe no relationship between methadone concentration and EtG concentration.

4.3.4 Discussion

4.3.4.1 Recent Alcohol Consumption Biomarkers (EtG and EtS) in Patients Receiving Methadone Substitution Maintenance

The aim of study 2 was to investigate the application of the new recent alcohol urinary biomarkers EtG and EtS during methadone substitution treatment. In total, 60 participants were interviewed at baseline and a total of 138 urine samples were collected after further interviews within four weeks of the first interview.

The main findings indicated that EtG and EtS were both detected in this group of patients. In our study, 100 out of 138 samples were positive for both EtG and EtS (41%). The use of direct alcohol biomarkers has been of increasing interest and has been investigated in recent studies; however, patients were mainly alcohol dependents and the biomarkers were aimed at investigating abstinence (Wurst et al., 1999).

At baseline the findings of the study indicated a high level of agreement between positive EtG and EtS urine levels and self report in the past 24 hours or past week (Cohen's Kappa 0.169). Wurst et al. measured urinary EtG and EtS in patients receiving methadone substitution and were able to detect positive results in 15 patients out of 26 who reported alcohol use in the past 7 days. Reassuring the patients about the confidentiality of the results may influence the high agreement between EtG/EtS levels and reported alcohol use in the study. However, further analysis of the samples collected indicated that several patients with positive EtG and EtS levels did not report any alcohol use in the past 24 hours (n = 29). These findings, however, might be due to consumption of alcohol before the 24 hour period. Studies have reported the detection of EtG for up to 4 days after alcohol consumption among participants who reported drinking heavily (Seidl et al., 2001). Another explanation

for this observation is that EtG might accumulate in prolonged drinking (Schmitt et al., 1997). Therefore, although EtG and EtS can be utilised in detecting alcohol use among patients receiving methadone, positive results may indicate a longer time-frame in which alcohol was consumed. However, no studies have reported detecting EtG or EtS for longer than 7 days.

The relationship between EtG and EtS was investigated using a Spearman's Rank Correlation, which indicated a strong association between the two analytes (R^2 = 0.95). Further investigation using linear regression was conducted to allow more accurate visualisation of the relationship. Linear regression presented in a scatter plot indicated that concretions with lower values had a better relationship. A histogram was also used to indicate the range of the ratio measured in the samples, which had a mean of 2 and ranged between 0.39 and 24. One sample had an extremely high ratio (24) and further investigation indicated that the sample was only negative for EtS. In total there were eight samples that were only negative for EtS and another eight that were only negative for EtG. In the case of a negative value for EtS, this could be attributed to the false positive of the EtG, which has been documented in previous studies (Dahl et al., 2002) as arising from urine bacterial contamination. However, the studies have shown that it is only EtG that degrades through bacterial hydrolysis and not EtS. This is related to the presence of the B-Glucuronidase in most E.coli strains, which is the most common pathogen in urinary tract infections, indicating that there is a risk of false EtG results (Ronald et al., 2002). However, EtS is not affected by bacterial hydrolysis and due to the similar time course in detection, measurements of both EtG and EtS have been recommended to avoid false positives (Helander and Beck, 2004; Helander and Beck, 2005).

In those participants who screened positive for both EtG and EtS, the value of EtG and EtS concentrations had a wide range. The mean urinary concentration was 115.11 ± 199.87 mg/L (range 0.07 - 1000 mg/L) and the mean urinary EtS concentration was 49.32 ± 82.46 mg/L (range 0.70 - 445 mg/L).

These findings correspond with previous studies indicating that EtG levels are usually higher than EtS levels. Both our studies, and other studies of EtG and EtS in blood and urine (Helander et al., 2009) indicated that levels of EtG were higher than the corresponding levels of EtS, even when the levels are corrected for their different molecular weights, with molar EtG/EtS ratios reported as 1.7. Only one study (Helander et al.) in urine showed higher concentrations of EtS than EtG .

Previous studies have mainly been in healthy participants (Wurst et al., 2006; Lostia et al., 2013). Studies investigating alcohol patients have reported a higher EtG concentration of 130 mg/L and a higher concentration of 110 mg/l for EtS. However, these levels may be due to the objective of the study, which was to look at alcohol levels during alcohol detoxification (Helander et al., 2009). Slightly higher results were reported by Dahl et al. (2011) in a group of patients who received methadone substitution: altogether, 26% of the urine samples from 12 of 24 patients tested positive for EtG (0.5–434 mg/l) and/or EtS (0.1–87 mg/l).

In this study, the value of EtG and EtS were given the absolute amount of (mg/L) and were not normalised to creatinine content. Although correction to creatinine is a common practice to compensate for diluted urine samples (Bendtsen and Jones, 1999), studies have indicated that the inter-individual variability is noted even after the correction in excretion (Goll et al., 2002), and even when the same dose of alcohol has been administered (Sarkola et al., 2003).

Cut-off values have been established in clinical studies to avoid false positive results (exposure to mouth washes and sanitizers). However, there is no reporting limit established for urinary EtG and EtS. In this study, a threshold of 0.05 mg/L for both EtG and EtS was used. Eight samples were found to be EtS positive only. All the samples were from patients who scored less than 8 on the AUDIT. This can be an indication of alcohol consumption earlier in the week and possible under-reporting of alcohol consumption.

One aim of the study was to identify the use of alcohol using a self-report screening tool compared to objective measures. Our findings indicated that AUDIT was successful in screening patients with problematic alcohol consumption. The use of screening tools to identify alcohol use among patients receiving methadone substitution treatment has been reported in the literature in Chapter 2. Teplin et al. (2007) used the MAST questionnaire and were successful in finding that 76% of patients presenting to clinics in the province of Ontario presented with alcohol problems, of which 9% suggested alcohol dependence. However, the author reported that the high prevalence of reported alcohol consumption may have been influenced by the reassurance that the data will be confidential. Another study by Senbanjo et al (2007) used the AUDIT among methadone patients to evaluate the impact of excessive alcohol consumption on quality of life. Although the evidence of alcohol consumption among patients receiving methadone treatment has been well established, there is a lack of clear guidance and policy for alcohol screening and testing in methadone treatment clinics. The clinic from which the participants in our study were recruited did have a protocol in place: patients who had been identified as having problematic alcohol consumption were required to be screened daily using a breathalyser. However, it is likely that some patients who do not present with alcohol-

related problems would remain undetected in the absence of a screening procedure. Thus patients who drink in a binge pattern risk being undetected. The results also indicated that some of the patients who scored less than 8 on the AUDIT, i.e., those who were low risk, had urine samples that were positive for EtG/EtS, indicating that they had consumed alcohol in the past 72 hours. This is consistent with findings by Gossop et al. (2003). The findings of our study indicate that a high number of patients who are presenting with alcohol problems scored 20 or more on the AUDIT, indicating the likelihood that these patients are alcohol dependent.

This evidence of problematic alcohol consumption in individuals on a methadone substitution programme is consistent with previous studies, where up to 50% of heroin dependents in receipt of methadone treatment also had alcohol-related problems (Rittmannsberger et al., 2000; Hubbard et al., 1986; Ottomanelli et al., 1999). In the United States, the Drug Abuse Treatment Outcome Studies (DATOS) indicated that of all methadone treatment patients, between 20% and 50% present with alcohol related problems (Hubbard et al., 2003). The concurrent dependence on alcohol among patients receiving methadone substitution treatment has also been highlighted in the literature. However, studies have indicated that the numbers are usually lower than anticipated and alcohol dependence is more likely to be present in patients who also found to be cocaine dependent and are therefore polysubstance users (El-Bassel et al., 1993; Senbanjo et al., 2007). Using AUDIT as a screening tool, of the patients scoring 8 or more, 35% (n = 21) scored 20 or more, indicating alcohol dependence.

Although more male patients presented with hazardous or harmful alcohol consumption, the excess was not significant. This is also consistent with previous

findings of Grella et al. (1999), suggesting that in a methadone maintenance clinic, male patients are more likely than female patients to misuse alcohol.

Of the 138 samples, 100 were positive and 22 were negative. Eight samples were positive for EtG only and another eight were positive for EtS only. Previous work (Dahl et al., 2011) has expressed concern regarding false-positive results due to accidental exposure, such as the use of hand sanitizers or alcohol mouthwash. The introduction of a cut-off between 100-250 ug/L to exclude any accidental exposure of alcohol and to measure EtS simultaneously would limit this false positive. The potential of false positives has also been investigated in in vitro formation and degradation of EtG and EtS, highlighting the potential of causes due to urinary tract infections. One study indicated a complete degradation of EtG within 3-4 days by E.Coli; however EtG remained stable for up to 11 days (Helander et al., 2007).

The findings of the self-reports of alcohol were more consistent in patients who scored more than 8 in the study compared to patients who scored less than 8. This might be explained as due to the fact that these patients were more likely to be breathalysed and were informed that the data were confidential. This is consistent with previous research, demonstrating that EtG has high specificity by Wurst et al. (2003).

Findings also indicated that both EtG and EtS were significantly correlated with AUDIT scores. This indicates that AUDIT could be used as a reliable participantive measure of alcohol use. However, some participants who scored below 8 presented with positive urinary EtG and EtS, indicating recent consumption, possibly low amounts of alcohol. Even low or infrequent alcohol consumption on a methadone treatment programme has the potential to be problematic, and in some instances, it

can be fatal. The importance of monitoring participants who consume alcohol, even those who consume alcohol in a less overtly problematic fashion, is still important, because episodic alcohol consumption in this group can be risky. No previous studies have investigated the correlation of AUDIT and EtG or EtS levels. These findings are consistent with Wurst et al.'s results (2008a): using the total score of the AUDIT, Hair EtG confirmed 10 more cases positive for alcohol, and of the 14 participants who reported no alcohol intake during the previous 7 days, 4 were Urine EtG positive.

4.3.4.2 Clinical Implication

The data from the present study provide supportive evidence of the usefulness of measuring recent alcohol biomarkers urinary EtG and EtS in patients receiving methadone maintenance. The results indicated that these new biomarkers were able to detect alcohol use in both patients presenting with problematic alcohol consumption as well as patients who are not routinely monitored by the clinic but who however might drink in a binging pattern or use alcohol less frequently.

The results also indicate a high correlation between biological markers EtG and EtS in urine and the AUDIT positive scores. Therefore dual use of objective and participantive measure can be utilised to provide a more sensitive and specific means of identifying alcohol recent consumption. This can be useful at the beginning of the treatment where patients who consumed alcohol excessively can be detected early in the treatment and receive the intervention, which addresses a range of alcohol problems. The application of these tools are also useful during maintenance treatment, especially in cases where patients are not attending daily or have missed their doses for one or more days or over the weekend. It is also important to note that pattern of

alcohol consumption affects the methadone metabolism as discussed in Chapter 6. Acute alcohol consumption leads to methadone interaction and increases the potential of overdose. This also highlights the potential application of EtG and EtS, which, in comparison to liver biomarkers for chronic alcohol consumption, can detect cases of binge drinking.

In conclusion, alcohol consumption can be problematic among patients receiving methadone. Alcohol use may hinder methadone treatment outcomes and in some cases can cause overdose and death. The use of traditional biomarkers do not directly measure alcohol. Liver enzymes are difficult to interpret in patients receiving methadone treatment due to the high prevalence in this population of hepatitis C infections. Substitution treatment represents a common challenge. In some cases, patients present with concomitant alcohol dependence that leads to negative treatment outcomes including disengagement with treatment (Stenbacka et al., 2007). The results of this study indicates that urinary EtG and EtS testing among patients receiving methadone substitution was successful in objective screening for recent alcohol use. Therefore EtG and EtS can be considered valuable objective tools to assist with obtaining a fuller clinical picture during methadone substitution treatment.

4.4 Study 3: An Investigation Exploring the Use of the Breathalyser during Methadone Treatment

4.4.1 Background

This study investigated breathalyser readings in patients receiving methadone maintenance who were on fixed daily dosing regimes and who met criteria for hazardous/harmful alcohol use. The aim of the study was to help establish whether the

clinic's breathalyser cut-off limit for alcohol use among clients receiving methadone replacement was a suitable measure of their alcohol consumption.

4.4.2 Methods

Validated questionnaires were administered, including the AUDIT (screening), LDQ (a measure of dependence), and the SOWS and SAWS, both measures of withdrawal (see Appendix F). Questions related to alcohol included family history, amount consumed on a typical day and time of last consumption when collecting methadone. A urine sample was collected after the interview to measure EtG and EtS levels. All samples were collected in 20mL universal tubes at trough, i.e., before administration of the daily dose of methadone. Participants were provided with a urine bottle and asked to void unobserved. The urine samples were frozen in the laboratory at -20° C until analysis. At the end of the interview, each participant was assigned an appointment for his or her next interview. The whole procedure, including the structured interview, took approximately 30-40 minutes.

4.4.3 Results

4.4.3.1 Demographic Information

Table 8-1 summarises the demographic information regarding the 23 study participants. Since the data were measured categorically, frequency and percentage summaries were used. Almost half of the participants were either self-referred (21.7%, n = 5) or were referred by a consultant (21.7%, n = 5). More than half were White British (73.9 %, n = 17) and only two were currently employed (8.7%). The majority (73.9%, n = 17) of the patients had no formal educational qualifications,

while four (17.4%) had GCSE or O-level qualifications. The mean age was 41.5 years old (range 29-57). Almost half (53.3%, n = 16) were in the age range 38 to 45 years.

Further demographics related questions were collected from the subjects' medical records. Of the 23 subjects, only four were receiving benefits (17.4%). In terms of housing, most of the subjects' accommodation type was known (39.1%, n = 9): eight subjects lived in council accommodation (34.8%) and four reported being homeless (17.4%). Only one subject reported owning property and one reported privately renting. More than half of the subjects reported living alone (56.5%, n = 13). Participants were asked about their driving history and results indicated only one participant out of the 23 reported having a valid driving licence. Five participants (21.7%) reported having previously held a licence and (21.7%) also reported being held for drink or drug driving. None of the participants reported driving to the clinic.

Table 4-34 *Frequency and Percentage Summary of Demographic Information in terms of sex, ethnicity, and referral route*

Demographic	Frequency (n)	Percent (%)
Sex		
Female	7	30.4
Male	16	69.6
Ethnicity		
White British	17	73.9
Irish	3	13
Caribbean	1	4.3
Other	2	8.7
Referral route		
Came by self	5	21.7
Referred by GP	1	4.3
Sent by family	3	13
Transfer from prison	3	13
Referred by a consultant	5	21.7
Other	6	26.1

Table 4-35 *Frequency and Percentage Summary of Demographic Information in terms of employment, benefits, accommodation, living status, and education*

Demographic	Frequency (n)	Percent (%)
Employment		
Employed	2	8.7
Unemployed	16	69.6
Other	5	21.7
Benefits		
Receives benefits	4	17.4
Do not receive benefits	19	82.6
Accommodation		
Council	8	34.8
Owned	1	4.3
Homeless	4	17.4
Rent	1	4.3
Not known	9	39.1
Living Status		
Lives alone	13	56.5
With a partner	2	8.7
With family	3	13.0
With a friend	1	4.3
Not known	4	17.4
Education		
No formal qualifications	17	73.9
GCSE / O-Level	4	17.4
Vocational qualifications	2	8.7

4.4.3.2 Medical Health

Participants completed two scales adapted from the Treatment Outcome Profile (TOP) relating to health, which included a subjective rating score between 0-20 for physical and psychological health status. The mean subjective physical health score was 8.7 ± 4.2 (range 3-18), whereas the mean subjective score for psychological health was 10.1 ± 5.2 (range 1-20).

From the data collected from the ePJS (electronic patient journey system) clinical records, more than half of the patients tested negative for HIV (n = 14, 60.9%); the remaining nine subjects had no evidence of testing having been carried out. All subjects had been tested for hepatitis-C: 10 had tested positive (43.5%), nine tested negative (39.1%) and for four subjects the results were unrecorded and thus unknown to the researcher (17.4%). All 23 participants had been tested for hepatitis-B. Almost half of the subjects were immunised against hepatitis-B (n = 13, 56.5%). Nine subjects were negative (39.1%) and only one subject tested positive for hepatitis-B. Hepatitis-B vaccination status indicated that only eight subjects were found to have completed the vaccination course (34.8%).

Thirteen subjects (56.5%) reported being prescribed only methadone. Of the ten subjects (43.5%) who reported that they were being prescribed another medication along with methadone, four (17.4%) were prescribed antidepressants, five (21.7%) were prescribed more than two medications, and one was prescribed an antipsychotic.

4.4.3.3 Methadone treatment

Participants were asked about the year in which they were first prescribed methadone, and responses ranged between 1999 and 2014, indicating that some subjects had first been prescribed methadone more than 12 years previously. The mean dose of methadone prescribed was 61.3 ± 18.4 mg/day (30 – 100 mg/day). Almost all of the subjects collected their methadone prescriptions daily (n = 20, 87%). Only one subject collected his dose from the clinic weekly and another subject reported collecting his dose from the clinic once every two weeks. More than half of the subjects reported missing a dose in the last week (65.2%); four reported never missing doses (17.4%) and only three reported missing their methadone dose (earlier) in the past month.

Almost all of the subjects reported that they found themselves increasing their alcohol intake when they missed their methadone dose (82.6%). Eleven subjects reported using illicit methadone (47.8%); however, they also reported this as infrequent.

4.4.3.4 Alcohol and Illicit Drug Use History

Table 4-36 summarises the results of a t-test analysis conducted on female and male alcohol and illicit drug use history in subjects in the study.

Table 4-36 *T-test analysis of female and male alcohol and illicit drug use history in subjects in the study*

Demographic	Sample (n= 23)	Male (n= 16)	Female (n= 7)	t	df	p-value
Family history						
Alcohol dependence (yes/no)	17/6	11/5	6/1	-0.83	21	0.39
Received alcohol dependence treatment (yes/no)	6/17	5/11	1/6	0.83	21	0.39
Drug dependence (yes/no)	13/10	9/7	4/3	-0.38	21	0.96
Received drug dependence treatment (yes/no)	12/11	8/8	4/3	-0.3	21	0.75
Mean age of first use (SD*)	21 (5)	20 (5)	23 (20)	1.23	21	0.23
Problematic Substance (N, %)						
Main problematic substance	Heroin (19, 82)	13	6	0.06	20	0.95
Second problematic substance	Crack (13, 56.5)	9	4	1.06	19	0.32
Third problematic substance	Alcohol (14, 60.9)	11	3	1.24	20	0.17
Route of Administration (N, %)						
Smoking	(6, 26.1)	5	1			
Injecting	(15, 65.2)	10	5	0.92	21	0.37
Oral	(2, 8.7)	1	1			
State of Injecting (N, %)						
Currently injecting	(12, 52.2)	9	3	-0.91	21	0.92
Not injecting	(9, 39.1)	6	3			
Never injected	(2, 8.7)	1	1			
Injecting in the past 28 days N (%)						
Yes	11 (47.8)	8	3	0.32	21	0.77

*SD = Standard Deviation

The analysis found no significant differences between female and male participants with respect to alcohol consumption.

4.4.3.5 Illicit drug use

Table 4-37 summarises self-reported information on participants' illicit drug use.

Table 4-37 *Self reported data on illicit drug use*

Demographic	**Sample (n= 23)**
Heroin use in the past 24 hours (yes/no)	17 (73.9%) / 6 (26.1%)
Amount in grams in the past 24 hours (g)	0.37 ± 0.27 (0.5 – 1.2)
Average amount per day in pounds (£)	18 ± 12 (5 – 60)
Use in the past month (yes/no)	19 (82.6 %) / 4 (17.4 %)
Number of days used	18 12 (2- 28)
Crack use in the past 24 hours (yes/no)	12 (52.2%) / 11 (47.8%)
Amount in grams in the past 24 hours (g)	8.4 ± 28 (0.03 – 100)
Average amount per day in pounds (£)	18.8 ± 22.9 (5 – 100)
Use in the past month (yes/no)	16 (69.6%) / 7 (30.4%)
Number of days used	9.6 ± 9.4 (28 – 9.6)
Cannabis use in the past month (yes/no)	5 (21.7 %) / 18 (78.3%)
Benzodiazepine use in the past month (yes/no)	7 (30.4 %) /16 (69.6%)

Seventeen subjects (73.9 %) reported using heroin in the past 24 hours, while 12 (52.2%) reported using crack cocaine. The use of heroin and other illicit drugs indicated a high level of treatment non-compliance. Seven subjects (30.4%) reported using benzodiazepine, and one subject reported using up to seven 10 mg diazepam pills per day.

4.4.3.6 Alcohol consumption behaviour and related data

All participants reported drinking alcohol regularly, and all except one reported drinking in the past month (95.7%). Nineteen participants (82.6%) reported drinking

strong beer, with 14 (60.9%) reporting drinking alcoholic beverages with ABV of 9%. Of the subjects who reported drinking, the mean number of alcohol units consumed in the past month was 627 ± 377.2 units of alcohol (4 – 1260) units of alcohol. All except two of the subjects reported drinking in the previous 72 hours. The mean number of units of alcohol consumed in the previous 24 hours was 35.4 ± 53.6 units of alcohol (range 4.2 – 270 units). Some subjects reported that their last drink had been taken in the late evening (between 9pm and 12am), while 12 participants (52.1%) reported that their last drink had been taken during the early hours of the morning. Thirteen participants reported drinking before consuming their daily dose of methadone (56.5%), while nine (39.1%) reported drinking after receiving their methadone dose. The mean score for the Alcohol Use Disorders Identification Test (AUDIT) was 25.1 ± 9.8 out of a possible 40 points (range 1 – 40 points).

4.4.3.7 Breathalyser Data

All participants reported being breathalysed when they attended the clinic to receive their methadone treatment. Nineteen participants (82.6%) reported being breathalysed at each attendance, two (8.7%) reported being breathalysed at about one-half of their attendances while another two reported that they were rarely breathalysed.

Participants were asked to recall their most recent 'failed' breathalyser reading (according to the guidelines in the Blackfriars clinic, > 0.39 g alcohol/l is considered failing), and the mean score was 0.51 ± 0.14 g alcohol/l (0.34 – 0.89 g alcohol/l). Three subjects reported blowing over the cut-off limit on the day of the interview (13%). Four participants reported failing the breathalyser test the day before the interview (17.4%) and four reported failing the test in the previous week. Four

reported failing the breathalyser test in the last year, and six participants (26.1%) reported never failing their breathalyser test. Only one reported failing it (earlier) in the same week of the interview and one reported failing the test in the last month.

Fifteen of the participants (65.2%) reported changing their alcohol consumption behaviour to avoid failing the breathalyser test. Of these, eight participants (34.8%) did not drink alcohol in the morning before visiting the clinic. Four participants (17.4%) reported that they tried to stop drinking early the previous evening in order to avoid failing the breathalyser test, and two (8.7%) reported having chosen drinks of lower ABV than they typically consumed.

4.4.3.8 Withdrawal Symptoms

Participants were also asked to complete the Subjective Opiate Withdrawal Scale (SOWS) and Subjective Alcohol Withdrawal Scale (SAWS). The SOWS measures the presence and intensity of symptoms of opiate/opioid withdrawal and the SAWS measures symptoms of alcohol withdrawal. Both are self-administered ten-item questionnaires for which each item is rated on a four-point scale (0 = none, 1 = mild, 2 = moderate, 3 = severe) (see Table 4-38).

Table 4-38 *Results of SOWS and SAWS scores*

Subject number	SOWS score	SAWS score
1	0	0
2	30	0
3	3	6
4	30	30
5	30	30
6	0	0
7	13	29
8	0	0
9	5	8
10	2	6
11	16	21
12	0	23
13	12	15
14	0	2
15	27	16
16	0	0
17	22	18
18	0	15
19	14	9
20	0	9
21	9	13
22	3	11
23	0	1
Mean ± SD	11.3 ± 10.1	9.3 ± 11.2

The mean SOWS score was 11.3 ± 10.1 (0-30), slightly higher than the mean SAWS score of 9.3 ± 11.2 (0-30). A one-sample test analysis indicated that there was significant difference between SOWS mean scores and the SAWS mean scores (t= 4.5, df = 21, p = 0.01). Pearson Correlation analysis indicated a weak correlation

between these scores (r = 0.56, df = 21, p = 0.01). Table 8-5 summarises the results of each participant's score for SOWS and SAWS.

4.4.4 Urinary Biological Analysis of EtG and EtS

Twenty of the participants provided urine samples that were analysed for EtG and EtS using LC/MS. The samples were collected at the end of the interview and were stored at a -20 C freezer in the laboratory until analysis. The mean EtG urine concentration was 30 ± 39.8 (0 –133) and the mean EtS urine concentration was 12.8 ± 15.8 (0 – 60.7). Of the 20 samples analysed, 17 (73.9%) tested positive for EtG and EtS. The mean EtG:EtS ratio was 2.2 ± (0 – 4.8). The ratios were highly correlated (r = 0.94, df = 18, p < 0.001) with a significantly linear relationship, as can be seen in the Figure 4-19 below.

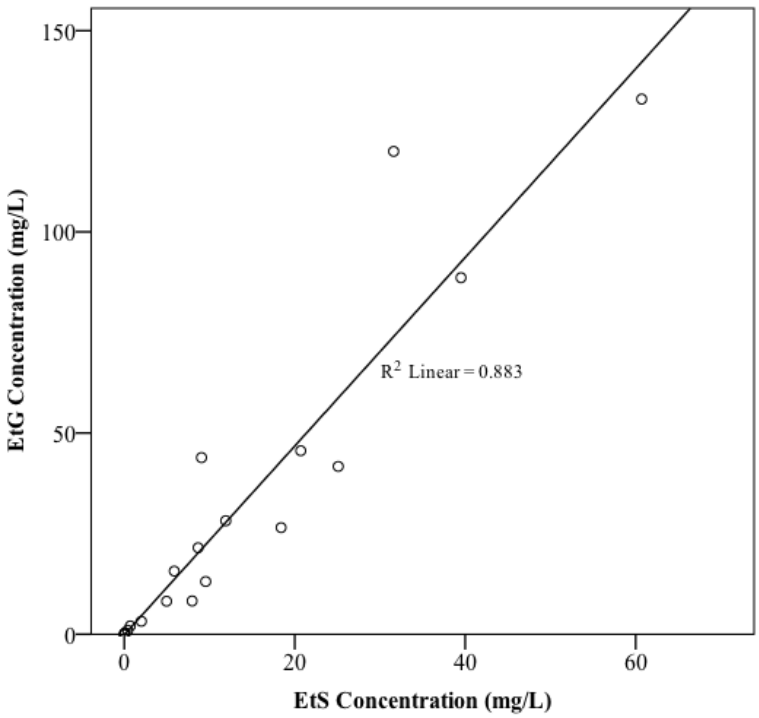


Figure 4-19 *Scatter plot of EtG and EtS urinary concentration (mg/L) in the study subjects (n= 20) with hollow circles presenting the relationship between EtG concentration and EtS concentration in urine. The line indicates a strong linear relationship (R^2= 0.88)*

4.4.4.1 Correlation between objective measures and subjective measures of alcohol use EtG and EtS concentration

The 17 samples that tested positive for EtG and EtS represent 85% of the 20 urine samples analysed. Given these data, Cohen's Kappa indicated a strong agreement between self-report regarding alcohol consumption in the past 24 hours and positive EtG and EtS analyses (Cohen's Kappa 0.61).

4.4.4.2 Breathalyser test

Eleven of the participants reported their breathalyser reading on the day of the interview, and a urine sample was collected and analysed for EtG and EtS. Table 8-6 summarises the descriptive statistics for these participants. Results indicate that only two participants (18.2%) tested positive for alcohol using the breathalyser (cut off above 0.39), as a result of which they were not prescribed their daily methadone dose. Compared with EtG EtS positive results, breathalyser positive results indicated a very weak agreement with self-report regarding alcohol use in the past 24 hours (Cohen's Kappa 0.10).

Table 4-39 *Descriptive Statistics for Hepatitis, Self- Reported Alcohol Intake, Alcohol Use Identification Test, and Biomarkers for Subjects Participating (mean, SD) summarises the descriptive statistics of the 11 subjects*

	Female (n = 4)	**Male (n = 7)**
Age Mean (SD)	40 (2.98)	41 (3.28)
Medications other than methadone (n)		
Yes	2	4
No	2	3
Name of other meds (n)		
Antidepressant	2	1
Mixed	0	3
Alcohol Percentage (%)	9	8.1
Units of Alcohol per Day	27	21.1
Units of Alcohol consumed in past month	683	600
AUDIT Score Mean (SD)	29.5 (5.19)	25.14 (12.4)
Breathalyser Reading	0.14 (0.1)	0.16 (0.2)
[a]Episodic heavy Drinking (n)	4	5
SOWS Score Mean (SD)	7.7 (10.4)	10.5 (5.2)
SAWS Score Mean (SD)	8.2 (8.6)	10.8 (10.1)
Hepatitis C (n)		
Positive	0	3
Negative	3	3
Not known	1	1
Hepatitis B (n)		
Positive	0	1
Negative	1	4
Immunised	3	2
HIV (n)		
Positive	0	0
Negative	2	4
EtG Concentration (mg/L) Mean (SD)	24.68 (23.37)	27.56 (48.09)
EtS Concentration (mg/L) Mean (SD)	9.53 (8.38)	13.38 (22.04)
EtG:EtS ratio Mean (SD)	2.61 (1.6)	1.5 (1.14)
EtG Positive (>0.05) (n)	4	5

AUDIT, Alcohol Use Disorders Identification Test; SOWS, Short Opioid Withdrawl Symptoms; SAWS, Short Alcohol Withdrawal Symptoms; EtG, ethyl glucoronide; EtS, ethyl sulphate.

[a]Defined as six or more drinks of alcohol on one occasion.

Unit consumed was corrected according to the time of last consumption in the past 24 hours according to the following equation: **Units consumed x (24- time) / 24**

Further analysis using breathalyser values indicated a good linear relationship (after time correction) between breathalyser scores and self-reported consumption in the past 24 hours, as shown in 4-20.

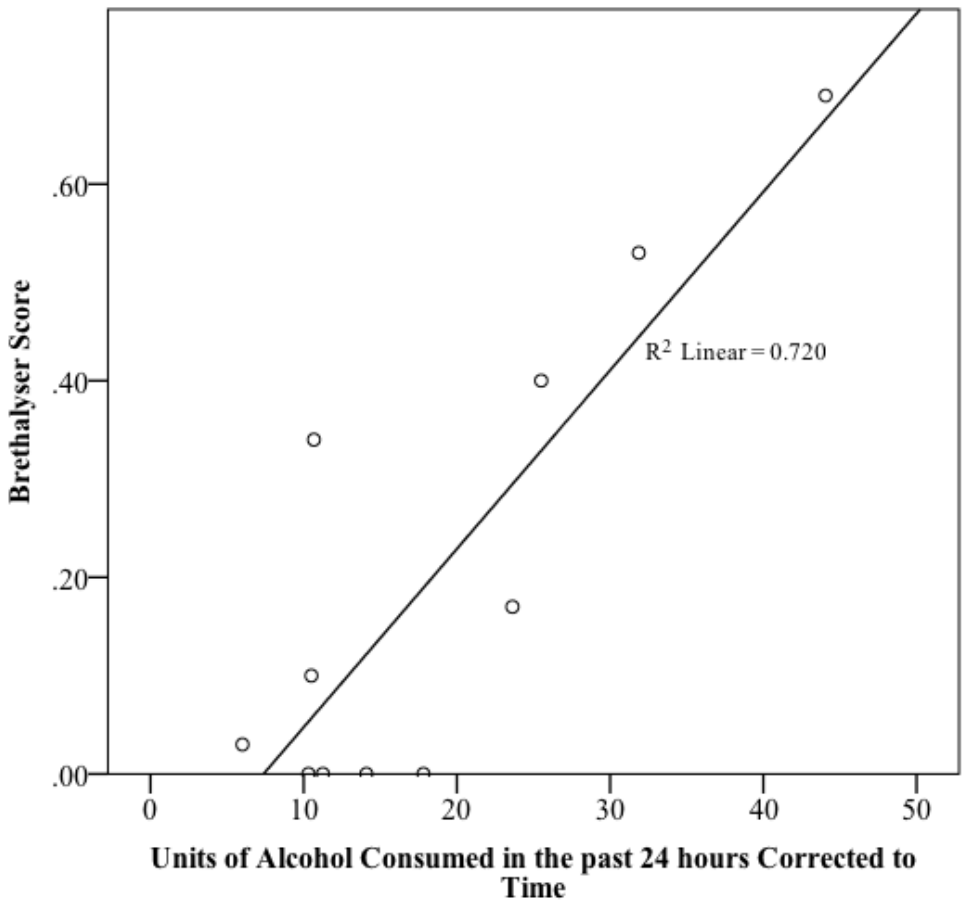


Figure 4-20 *Scatter plot with fitting line indicating a good linear relationship (R^2=0.72) with each hollow circle representing a subject's breathalyser reading against time corrected units*

4.4.4.3 EtG Concentration Cut-off Limit

Figure 4-21 presents the relationship between brethalyser test results and EtG concentration. Positive breathalyser results using 0.39 as a cut-off point are plotted against EtG concentration values after removing two outliers (two subjects with high levels of EtG concentrations and negative breathalysers). The cut-off point for EtG, therefore, is suggested to be between 26.5 and 28.2 mg/L.

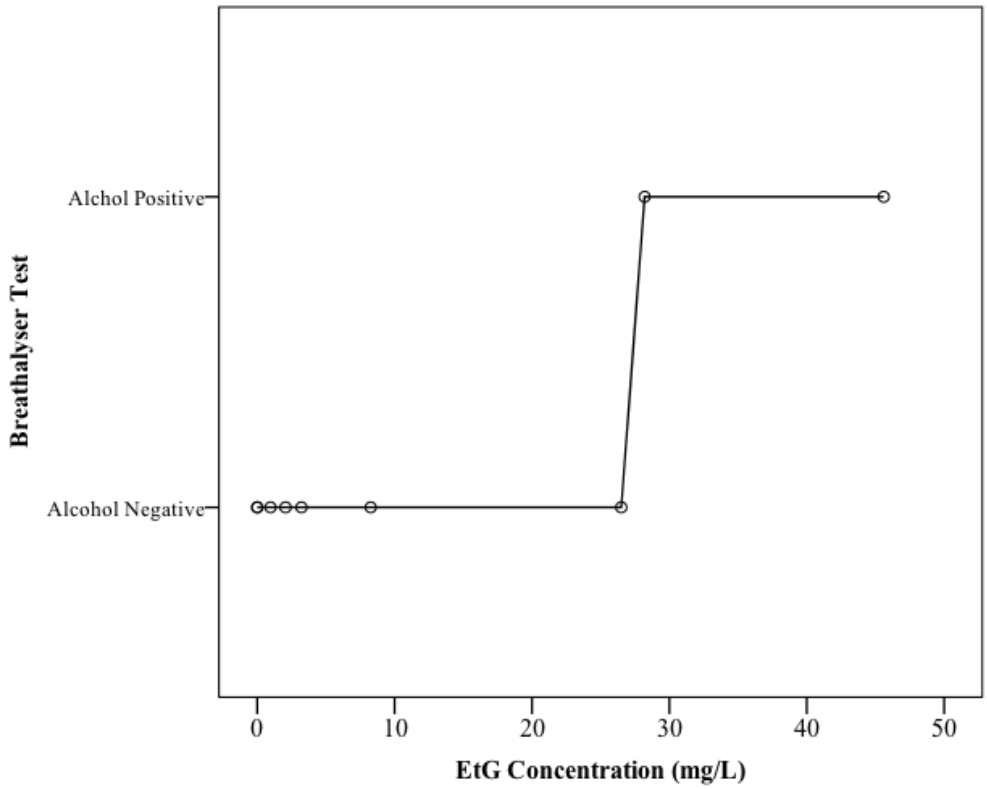


Figure 4-21 *Scatter plot of breathalyser test (alcohol positive or negative) against EtG concentration*

4.4.5 Discussion

The aim of this study was to examine the effectiveness of using alcohol breathalyser to monitor patients' compliance (which includes not presenting intoxicated) during methadone substitution treatment. Alcohol use among patients receiving methadone substitution is a form of non-compliance to treatment, as the patient is not taking the medication as instructed. Although, 'adherence' has replaced 'compliance' as a term to describe a broad range of behaviours that may affect how the patient takes his or her medication as recommended, compliance is the term that will be used in describing such behaviour in this thesis.

In this study, 23 of the 32 patients who were approached by the researcher participated. The patients were interviewed, data pertaining to demographics and alcohol consumption collected. Twenty participants provided urine samples, which were analysed for the recent alcohol use biomarkers EtG and EtS using LC/MS. Routine clinical monitoring for recent alcohol use is not well established in terms of guidelines or policy as part of methadone substitution treatment; the clinic from which the patients were recruited monitored patients presenting with problematic alcohol consumption using a breath alcohol test (breathalyser).

Breathlysers have been commonly used to measure recent alcohol consumption in clinical settings; more recently EtG has been approved as a marker for recent alcohol consumption. The main findings indicated that the breathalyser was sensitive in detecting the units consumed when corrected against time.

Hazardous or harmful alcohol use (as defined by AUDIT score) during methadone substitution treatment is difficult to establish because data are frequently collected using self-reported instruments. The use of more objective measures for recent

alcohol consumption has mainly been achieved using breathalysers, which were suggested as form of contingency management in the early days of developing methadone maintenance programs (Kreek et al., 1973). The use of biological tools to monitor recent alcohol consumption routinely in this cohort of patients has only relied on breathalysers. In this study, participants recruited from an outpatient facility (Blackfriars Road Clinic) were required to be breathalysed by the clinical nurse or the keyworker who was dispensing at the time. Patients were aware of the clinic's protocol and that methadone would not be dispensed if the breathalyser reading was 0.39 or over, as failing the breathalyser test is a sign of non-compliance. Other clinics use a breathalyser cut-off score of 0.35 or above (there is no scientific justification for these cut-off scores; they are just set slightly above the drink-driving limit). Positive EtG and EtS urine concentrations were determined using the King's College Biochemistry Laboratory cut-off levels, which were above 0.05 mg/L for both EtG and EtS.

Characteristics of the 23 participants suggested that they were typical of the total treatment population in South London. For instance, the mean age of the subjects was 41 years (29-57), 69.6% male and 73.9% self-described as White British. This is consistent with current studies investigating opiate dependents in South London (Alves & Winstock., 2012). The majority of participants in the nationwide sample for the National Treatment Outcome Study also described themselves as White (Gossop et al., 1997). The mean age of the group, moreover, is representative of patients presenting with severe problematic alcohol use while on methadone substitution, although compared to earlier studies the mean age reported is slightly higher. Best et al. (2000), for example, report a mean age of 36.4. Previous studies have established that patients' characteristics such as employment, education, psychological health and

history of criminal activity are linked to positive treatment outcomes and patients with good psychological support are more likely to benefit from methadone substitution treatment (Farrell et al., 1994).

The study participants all presented with problematic alcohol consumption, a factor that is frequently present in patients receiving methadone substitution treatment (Hunt et al., 1986). However, 82.4% of the patients in the study scored > 20 on the self-reported AUDIT instrument, which indicates a likelihood of alcohol dependence. It is important to distinguish between harmful drinking and alcohol dependence, which is in line with the ICD-10 (WHO 1992) definition of alcohol depdence. The use of AUDIT was able to help identify the severity of alcohol use problems among the population studied; however, it is apparent that the choice of diagnostic instrument and cut-off scores can contribute to differences in the rates of prevalence reported because it relies on self report and although there is a chance of underreporting, studies have indicated that drug and alcohol dependent patients have shown reliability in reporting their information (Del Boca & Noll, 2000). Patients were also reassured that the data collected would be anonymised in the study and that the researcher would not report back to the clinic.

Almost all participants reported drinking in the past 24 hours. Based on their reports, the amounts frequently exceeded the levels recommended by the UK Department of Health for any 24-hr. period, which are 16-24 g for females and 24 - 47 g for males. Moreover, all but three of the participants revealed that they had previously failed a breathalyser test. Although almost half of the patients revealed that they would start drinking alcohol after they had consumed their daily dose of methadone; they also reported the introduction of habits to avoid breathalysing positive for alcohol. Some

patients reported stopping drinking earlier than usual in the evening on the previous day, while others reported switching their drinking to alcohol of lesser strength. These reports of changing alcohol habits indicated that the use of breathalysers to monitor alcohol consumption was having an impact as a means of contingency management which involves a systematic application of positive reinforcement, and in this case the positive reinforcement would be receiving the methadone dose (Weaver et al., 2014), The use of breathalysers as a means of contingency management was suggested in earlier studies (e.g., Kellogg et al., 2005). This indicates that although the patients were compliant in one sense by not presenting to the clinic with intoxicating levels of alcohol (defined >0.39 using alcohol brethalyser), patients did consume high levels of alcohol soon after they received their methadone dose and were not compliant in that way. Therefore problematic alcohol use, along with non-compliance in the form of patients mixing alcohol with methadone, does not seem to be eliminated using breathalyser monitoring, instead patients consume alcohol at a time which correlates when methadone starts reaching its peak level in blood which is between 2.5-4 hours (Eap et al., 2002).

From the results (65.2%) of patients reported that they regulate their alcohol consumption habits to avoid positive breathalyser readings. Some revealed they would stop drinking alcohol earlier in the evening or choose a less strength alcohol enable to pass their daily alcohol breathalyser test. This can be explained as alcohol is eliminated by a rate of 0.1/g/kg per hour (Jachau et al., 2004). Therefore even an intake of an 8.7 units of alcohol will not be detected in breath after 9 – 10 hours.

This has been consistent with the results indicating that patients breathalyser score was highly correlated with the units of alcohol consumed when corrected to time.

Previous studies have investigated other biological markers for alcohol consumption compared with breathalyser results. Wurst et al. (2008b) conducted a study to compare several alcohol biomakers including recent direct ethanol biomarkers EtG with ethanol breath. The study results indicated that out of 146 urine samples, 14 samples were EtG positive while only one participant yielded a positive alcohol breath test. Findings from the Wurst study were consistent with our findings, indicating that the number of positive results with recent alcohol consumption using biomarkers such as EtG is higher than when using breathalysers.

Previous studies have compared breathalysers with other biological indicators including 5HTOL/5HIAA (Helander et al., 1999, Helander et al. 1996, Bendtsen et al., 1998). A study by Lahmek et al. (2012) found that 22 patients tested positive for EtG whereas only five (22%) tested positive with a breath alcohol test; however, 10 (45.5%) reported recent alcohol consumption. This high level of agreement between self-report and objective measures has also been supported by our study. Self-report agreement with positive objective measures was found higher in EtG and EtS concentration than breathalyser testing in our study. There was no significant difference between the breathalyser's measurements and the EtG and EtS concentration's results, but Cohen's kappa was lower when self-report agreement with an alcohol breathlyser test was compared to an EtG and EtS test, yielding a better agreement with the self report and EtG and EtS results (Cohen's kappa 0.3). This is due to a considerable number of positive EtG and EtS cases with positive self-reports of drinking. The results are not consistent with the study conducted by Wetterling et al. (2014), which indicated that the self-report agreed more with breathalyser data (Cohen's kappa 0.79). However, this difference could be due to the nature of the study, which intended to investigate abstinence in a group of alcohol dependent

patients after a weekend. Our study was investigating the ability of breathalysers to detect alcohol use when coming to the clinic to collect methadone maintenance. We found that urine tests to detect EtG and EtS biomarkers are more sensitive and more accurate than breathalysers in detecting alcohol use in the past 24 hours. Although the breathalyser was successful in detecting alcohol consumption in the past 24 hours, EtG and EtS urine concentration were more able to detect reported drinking. These findings are consistent with previous research by Dhal et al. (2011), who measured urinary EtG in outpatient treatment programmes for alcohol and drug dependence (n = 24). In 87% of the cases, the self-report information agreed with the EtG results (i.e. true positives and true negatives).

In another study by Wurst et al. (2008a), samples, collected from patients receiving methadone maintenance and alcohol consumption, were evaluated by measuring direct ethanol and self-report. Of the 14 subjects reporting use in the past week, (28.5%) were EtG positive.

Another factor in the importance of monitoring alcohol was the presence of patients presenting with Hepatitis C. Alcohol consumption may accelerate the progression of Hepatitis C and may result in incomplete treatment adherence and non-compliance due to other medical conditions (Peters & Terrault, 2002).

The presence of Hepatitis C, combined with chronic alcohol consumption, may lead to the increase of the progression of fibrosis (Wiley et al., 1998, Pessione et al., 1998) This was reflected in two patients who presented with severe liver problems and, due to accumulation of ethanol, they were considered an exception and the breathalyser cut off limit was increased to 0.60 for them.

The high number of negative breathalyser results maybe influenced by the cut-off limits implemented by the clinic. Other than contingency management or a purpose for controlling alcohol use among patients in the study, the use of a breathalyser cut off limit, which is slightly higher than the drink-driving limit, has no scientific basis. 0.35 is utilised in drink driving in the UK due to concerns about someone driving a vehicle with a level of intoxication above that limit. But these concerns do not necessarily apply in the clinical setting. Previous studies (Morrison et al., 1997) have investigated if MMT patients report to the DVLA regarding their methadone prescription. Morrison et al.'s study found that more patients had provisional licences than patients in our study, perhaps due to geographical considerations. In this study the cohort was taken from the inner city where there is less of a need to drive. Of the 23 patients in our study, none of them drove to the clinic; only one had a valid driving licence and five reported having an invalid licence. Therefore the link between the drink driving limit and the breathalyser test to monitor alcohol consumption is not necessarily a one with a pharmacokinetic basis.

The implication of this study is that breathlaysers are an effective biological tool for contingency management in patients presenting with alcohol problems (patients refrain from using alcohol, or change their behaviour so that they get their methadone dose). However, breathalysers are not necessarily suitable for monitoring compliance as they cannot necessarily detect drinking 24 hours before the breathalyser test because of the speed with which alcohol is excreted. The newly applied biomarkers EtG and EtS were more sensitive than the breathalyser: they would be able to detect alcohol use in the past 48 hours and even longer in chronic drinkers. In an attempt to indicate a relationship between the cut-off reading of the breathalyser and the EtG urinary levels, the study compared the EtG concentration when breathalysers were

positive and indicated that a cut-off level between 25 and 28 mg/L would be equivalent to 0.39. However, indicating a cut-off limit for EtG is beyoned the scope of this study. Further controlled studies are needed to establish the relationship between recent alcohol consumption biomarkers and breathalyser limits in relation to levels of low-risk, hazardous, harmful, or dependent consumption of alcohol.

The study had further implications as regards patients' alcohol consumption. The results also indicated that most patients (82.6%) found themselves increasing their alcohol intake when they missed their methadone dose. This phenomenon could be explained by the growing evidence of the link between the endogenous opioid system and the ability of alcohol release opioid peptides in regions associated with reward in the brain (Gianoulakis et al., 2001). This might also explain cases where patients who are under-medicated increase their alcohol intake. Interestingly, the mean dose of methadone prescribed was 61.3 ± 18.4 mg/day (30 – 100 mg/day). Eleven subjects reported using illicit methadone (47.8%); however, they also reported this as infrequent.

The presence of all these combinations is a clear indication of non-adherence to treatment. Although patients are coming to collect their daily methadone, they are not consuming it as instructed, which includes both refraining from alcohol consumption and not using on top. Although the breathalyser has been utilised as a useful means of contingency management, the addition of a more specific biomarker for periodical testing may in itself increase compliance.

4.4.6 Implications

The study's findings should encourage clinicans to consider more objective biological tools to assess recent alcohol consumption. While breathalysers a recurrently used to

monitor compliance, establishing a better understanding of what cut-off values should be implemented is important. Furthermore, the study indicated the importance of identifying patients who are presenting with dual dependence and the need to establish dual treatment management guidelines to address co-dependencies of alcohol and heroin.

The limitations of this study included the lack of distinction between gender in the scores collected, including the subjective measure using the AUDIT. However, it is important to note that breathalyser scores and EtG and EtS scores do not have gender specific cut-off values. The number of patients recruited in the study was small due to physical and time limitations. A wider study addressing these problems in more depth and scope could have a clearer sense of the issues involved.

4.4.7 Conclusion

As discussed, the problematic use of alcohol among patients receiving methadone treatment is well established. However, it is important for clinicians to identify instances of harmful, hazardous, and dependent consumption of alcohol in patients receiving methadone, and to be able to distinguish between different patterns of consumption based on biological tools. This study aimed to investigate whether breathalysers were useful in detecting compliance to treatment regarding avoiding the problematic consumption of alcohol.

Evidence from this study, through the comparison of objective biological tools, indicates that breathalysers are time dependent and, while useful in contingency management, are not as effective as biomarkers like EtG and EtS in identifying patterns of alcohol consumption outside of a 24-hour period.

Chapter 5 OVERALL DISCUSSION AND CONCLUSION

The aim of this thesis was to determine the usefulness of utilising biological markers to monitor patients' compliance during methadone maintenance treatment. The study investigated the metabolic ratio EDDP:methadone for monitoring compliance with methadone daily dose, EtG and EtS to monitor recent alcohol consumption, and alcohol breathalyser to determine its usefulness to monitor alcohol use during methadone maitenance treatment.

5.1 Main findings of this thesis

Methadone concentration and its inactive metabolite (EDDP) in urine have been studied previously (Kreek et al., 1973; Kell et al., 1994; Preston et al., 2004). The current study investigated urinary EDDP:methadone ratio in 60 patients after collecting a weekly sample for four weeks during methadone maintenance treatment. In total (n=138) urine samples were analysed and indicated EDDP:methadone exhibited inter-individual and intra-individual variability. This is consistent with previous studies, which explained that the reason for this variability could be owed to methadone metabolism where genetic variability in P-glycoprotein can exhibit up to 11-fold variations (Li et al., 2008).

The study has also investigated the relationship between EDDP and methadone and found they exhibit a positively correlative relationship, which supports the fact that methadone is metabolised to EDDP via N-demethylation (Eap et al., 2003). These findings highlighted the potential advantage of investigating EDDP in urine as a means of monitoring compliance. Because urine samples were collected from patients

receiving methadone maintenance treatment it was unlikely to find a negative methadone concentration unless a patient had an opportunity to adulterate the sample.

Furthermore, to control the variation observed in the ratio, the study investigated the ratio of EDDP:methadone in urine samples where several participants had the same dose: in some cases 40mg and in some cases 50mg. These ratios exhibited less variability between subjects; however, this drop in variability was more prevalent in patients with a 50mg daily dose than in patients with a 40mg daily dose. This could indicate that monitoring the ratio is better at high doses due to a more stable ratio between EDDP and methadone. However, of the patients who were noted as outliers, it was later found that they had been receiving methadone for less than three weeks, and because ratios are more variable during induction, this could establish the reason for this high ratio.

In a further attempt to control variability, the samples were investigated depending on alcohol consumption. Findings indicated that the EDDP:methadone ratio was significantly different between the two groups. This finding is consistent with previous studies indicating that chronic alcohol consumption has a direct effect on methadone concentration, a phenomenon that has been investigated by Clark et al. (2005). However the study failed to indicate a specific range that would help clinicians to indicate the relation to alcohol consumption.

Due to the high levels of variability observed in the study, it was not possible to establish the usefulness of the EDDP:methadone ratio; however, it will be useful for clinicians to measure EDDP as a mean of ensuring that the patients have taken their daily dose, as EDDP cannot be present in the urine unless methadone has been metabolised.

The study investigated the usefulness of measuring EtG and EtS to monitor compliance in 60 patients receiving methadone maintenance. From the 138 samples collected it was indicated that a few patients had reported not to have consumed alcohol but had positive EtG and EtS concentrations in their urine. This would indicate that EtG and EtS was successful in detecting recent alcohol consumption among this population. To our knowledge, only one other study has investigated the use of EtG and EtS in this population (Wurst et al., 2008). The findings indicated only a small number of patients presented with a low risk pattern of drinking. Both studies indicated that biological tools are more objective than self-report (AUDIT) as a means of monitoring compliance. However, EtG and EtS can be more relevant to be utilised as a clinical tool in alcohol dependence treatment compared to methadone maintenance treatment setting when abstinence is a required outcome. Both biomarkers can be used as an objective measure of relapse and can be considered to be more efficient than self-report, however they can also be used in combination which can have promising results. Furthermore, EtG and EtS can be more useful compared to breathalysers in detecting relapse in an alcohol dependence treatment setting. For example patients can go drinking for few days before coming reporting negative reading for alcohol consumption using the breathalysers leading to a negative outcome in long-term abstinence module.

The final study attempted to investigate the usefulness of the current practice of using breathalysers as a mean of objective measurement of recent alcohol consumption. The findings indicated that although the breathalyser was successful in managing the patients' recent alcohol consumption before attending the clinic, it was less specific than EtG and EtS in monitoring patients' compliance. This is consistent with studies that have investigated the breathalyser as a tool for monitoring alcohol consumption

in comparison with EtG and EtS, and found that in detecting alcohol consumptiom, breathalysers were less accurate as they depended on time, units consumed, and liver fucntioning (Wetterling et al., 2013).

The study indicated the importance of the use of breathalyser as a means of contingency management rather than an objective monitoring tool for recent alcohol consumption, and indicated that the biomarkers EtG and EtS would be more sensitive and specific tools in a clinical setting. The study has also highlighted the shortcomings of the use of breathalyser to detect intoxication in this population. For example participants presented with signs of intoxication including an odour of alcohol sometimes scores lower than the cut off limit and vice versa, where participants did not present with any signs of intoxication however did report drinking would score higher than the cut off limit. Furthermore, some participants reported the illicit use of benzodiazepine, which could lead to signs of intoxication. However, the use of breathalyser would have not been successful in detecting these sings which is an indication of the limitations of the use of the breathalysers in detecting intoxication signs which could lead to a fatal methadone, alcohol, and benzodiazepine interaction.

Findings investigating withdrawal symptoms indicated that the short withdrawal scale is not highly specific and when SOWS scale is compared with the SAWS scale few items were overlapping including insomnia and heart pounding. Some of the symptoms from both scales were also very similar in meaning such as feeling sick and nausea. Therefore, this research incorporated using more objective measures and biological indicators such as EDDP and Methadone urine levels to monitor clinical symptoms including withdrawal symptoms. The use of more ojective measures has

been previously suggested e.g. plasma cortical level (Bearn et al., 2001) or methadone plasma level (Wolff et al., 1990).

5.2 Limitations

The recruitment of the participants to the three studies required engaging with them during their daily visits to collect their methadone dose. The researcher had to be careful not to discrupt the participants' treatment due to the demands of the study. Collecting urine at trough, right before a patient takes his or her daily dose of methadone, can in some cases be difficult, due to a patient's agitation with withdrawal symptoms. Furthermore, participants were required to see the researcher three more times once a week after the initial interview. Owing to the sometimes chaotic nature of the participants' lives, it was difficult to obtain full commitment to the study in some cases and therefore not all participants provided a full set of urine samples. Some participants were also not able to void enough urine, which hindered the analysis of their samples.

Participants presenting with difficult behaviour were managed by the researcher in collaboration with their assigned keyworker. They were not recruited without consulting with their keyworkers to prevent any potential danger to the researcher as well as the participants. This can interpreted as a bias in the process of recruitment.

As discussed above, the questionnaire part of the study depended on self-report, including reporting illicit drug use and alcohol consumption. Two concerns were highlighted: the first was the potential for underreporting if the patient felt that the information divulged might affect his or her methadone script. Patients were reassured that the information collected was confidential, research-related, and would not be

shared with the staff at the clinic. The second concern was that some of the questions were related to drug use in the past month, which might have been affected by recall bias.

Although Study 1 and Study 2 aimed to collect patients at different stages of time, Study 3 collected data at one point of collection and there was no follow up to see whether drinking pattern were affected by time.

As with most qualitative research, the results generalisability and statistical significance are difficult to implement fully, due to the small sample size, especially in Study 3. Only patients who were breathalysed and who were on methadone were recruited, which proved diffiult ecause patients were only recruited from one site and only during the daily methadone collection hours of 10am and 12pm. The majority of participants in all studies were from a White British background, which makes it difficult to generalise the result with regard to the population as a whole. However, the results of this study are consistent with other research on methadone maintenance from Britain and America, which lends external validity to its findings.

Regarding urine analysis, adjustment to urine creatinine when measuring EDDP:methadone could have improved the variability; however, the method developed did not include creatinine measurement.

5.3 Future research

Since the introduction of methadone in the 1960's, it has remained the gold standard for heroin dependence treatment. Attempts to improve the current methadone maintenance services using objective biomarkers has been researched but not implemented as part of a clear management plan. This research attempted to identify

objective biological tools to assess patients' compliance during methadone maintenance treatment.

Although EDDP:methadone has presented with both inter-individual and intra-individual variability, it was observed that this variability reduced when analysed according to samples collected from patients receiving the same dose. Further research investigating the ratio in samples from patients with the same dose, and the addition of creatinine to adjust the ratio, could help reduce the variability and increase the potential of using the EDDP:methadone ratio as a specific biological tool to identify compliance.

Incorporating the use of biological tools in combination with self-report seems promising. However, this needs to be in line with clear guidelines and implementation of new policies for management of patients presenting with co-dependence that could be identified early on in the treatment. However, further research in cut-off values to identify low-risk, harmful, hazardous and dependent patterns of alcohol consumption, using EtG and EtS, in the context of patients presenting to methadone treatment, would be valuable in identifying patients with alcohol problems and therefore providing them with the most appropriate treatment modality.

Appendices

Appendix A

NHS
Health Research Authority

National Research Ethics Service

NRES Committee London - Fulham
HRA NRES Centre Manchester
Barlow House
3rd Floor, 4 Minshull Street
Manchester
M1 3DZ

Telephone: 0161 625 7821
Facsimile: 0161 625 7299

04 July 2012

Miss Basma Alharthy
Franklin-Wilkins Building, 150 Stamford Street
4th floor, room 4.124
London
SE1 9NH

Dear Miss Alharthy

Study title:	**Methadone and EDDP ratio in urine in patients receiving methadone treatment and its effect on treatment outcome**
IRAS Project Number:	**103596**
REC reference:	**12/LO/0762**

Thank you for your letter of 03 July 2012, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Appendix B

Institute of Psychiatry

at The Maudsley

KING'S College LONDON Founded 1829

University of London

Patient's Information Sheet Title of Research: Methadone and EDDP ratio in urine in patients receiving methadone treatment and its effect on treatment outcome

Name of Researcher: Basma Alharthy

Center No:

You are being invited to take part in a study, which is a part of a research project on methadone treatment for a PhD at King's College London. Before deciding to take part, you need to find out more about the reasons behind the project and what it will involve. If you have any questions, please ask them, and please feel free to take your time to decide whether you want to take part in the study.

What is the purpose of the study?

The most important aim of the study is to find out more about what happens to methadone in the body. We are going to do this by measuring the concentration of methadone and a metabolite of methadone (a break down product of methadone) called EDDP. By measuring the level of methadone and EDDP together we can find out how the drug behaves in your body and this will give us some information about treatment compliance.

Who can take part?

Anyone who is prescribed methadone or about to be prescribed methadone can take part. We are particularly interested in your help if you are:

1. About to start methadone treatment

2. Stabilized on methadone on a fixed daily dose

3. About to begin a programme of dosage reduction

It does not matter if you are taking other medication because we are also interested in the way in which different drugs change the way that methadone works. If you are also using other non-prescribed drugs, you can still participate in the study. You will need to sign a consent form saying that you agree to take part.

What are the benefits of taking part in the study?

You will be helping us to understand better the way in which methadone works in the body and help create better knowledge for clinicians about methadone treatment. Everyone who takes part in the research will be given a £10 phone card voucher after the first interview and urine sample collection. After that you will get a £5 phone card voucher for every further urine sample that you give.

Patient's Information Sheet. V2.0, 27-6-2012

Who will see my information?

Personal information will be stored confidentially on a computer. All information will be confidential, anonymously and in a secure office at King's College London. The project supervisor will ensure patient confidentiality.

The information collected will be given a code linking it to the collected urine sample but will be anonymised and will not be able to be linked to the participant. The information that you provide during the study will only be used by research purposes, for example (if you were reported using extra methadone on top of your prescription we would not feed this information back to the clinic. Any meetings with the consultant on charge will involve discussions about recruitment.

Who has reviewed the study?

Before the research starts it will have been approved by a Research Ethics Committee. In this case the review was carried out by the London-Fullham NHS Research Ethics Committee.

Name and contact details:

If you have any general questions about the study you can contact me using the details below

Chief Investigator: Miss Basma Alharthy Email: Basma.alharthy@kcl.ac.uk

If you require further details or if you feel that the study has been harmful to you in any way you can contact the Project Supervisor at King's College London using the details below for further advice and information:

Supervisor:

Dr Kim Wolff, Reader in Addiction Science, Institute of Pharmaceutical Science King's College London

Thank you for your time reading this information sheet.

!

You cannot take part in the study if you are pregnant or breastfeeding.

What will I have to do?

You will be asked to complete a self-report questionnaire asking questions about:

Your methadone treatment programme

Any withdrawal symptoms you may experience using a standard questionnaire called The Opiate Withdrawal Scale (SOWS)

Questions about other drug use, smoking and alcohol use

These questions will only take approx 10-15 minutes to complete. There will also be some more general questions about you and your current prescribed medications and non-prescribed drug use. You will be asked to provide one or more urine samples (20 ml of urine) before you receive your daily methadone dose depending upon the treatment programme that you are receiving.

How the study will work

We would like to recruit people on different methadone programmes so that we can find out as much as possible about the working of methadone. The groups below are the different types of treatment that we are interested in. You will need to tell us which one best describes your treatment.

Group A Group B Group C or non-prescribed drugs Group D Methadone reduction treatment (detoxification)

About to begin methadone treatment (dosage induction) Methadone maintenance treatment Methadone treatment but also prescribed other medication

The only difference between the groups is the number of times your will be required to give a urine sample. If you belong to group A, C or D we would like to see you only a daily basis and would like you to provide a urine sample before your usual daily dose for 14 days. If you are in group D we would like to meet you once a week for 4 weeks to collect a urine sample.

The urine samples will always be collected at the clinic just before you receive your daily dose of methadone. The urine samples will be stored in the secure laboratory at King's College London at -20oC until required for analysis. Once analysis has taken place the urine sample will be destroyed in accordance with the Human Tissue Act.

Can I pull out from the study?

Yes. If you feel unwell, uncomfortable, or unable to continue for whatever reason you are free to pull out of the study at any time. You do not have to explain the reason why you want to leave.

Appendix C

Consent Form v1.0, 24-4-2012

Institute of Psychiatry

at The Maudsley

KING'S College LONDON Founded 1829

University of London

Consent Form

Title of Research: Methadone and EDDP ratio in urine in patients receiving methadone treatment and its effect on treatment outcome
Name of Researcher: Basma Alharthy
Participants Identification No. for the study
Center No:

1. I confirm that I have read and understand the information sheet date _____ for the above study. I have had the opportunity to ask questions and I am happy with the responses given.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and it will not affect the service of care I receive now or in the future.
3. I understand and give permission for the researcher to have access to my records at _____clinic to collect demographic information for the purpose of study.
4. I understand that my data will be anonymous and my identity will not be revealed. I am aware that the data will be stored in security and destroyed after 6 months.
5. I am aware that if I become distressed, I can stop the study and would be encouraged to speak about it to my key worker.
6. I agree to take part in the above study.

Name of participant	Date	Signature
Name of person taking consent (If different from chief investigator)		
Chief investigator: Miss. Basma Alharthy Email: Basma.alharthy@kcl.ac.uk		
Supervisor: Dr. Kim Wolff Email: Kim.wolff@kcl.ac.uk		

Appendix D

Questionnaire v1.0, 24-4-2012

Institute of Psychiatry

at The Maudsley

KING'S College LONDON Founded 1829

University of London

Title of Research: Methadone and EDDP ratio in urine in patients receiving methadone treatment and its effect on treatment outcome

Name of Researcher: Basma Alharthy

Participants Identification No. for the study

Center No:

Please tick the stage of methadone treatment you are at:

I just started receiving methadone	
I have been receiving methadone	
I will start methadone detoxification	
I will start alcohol detoxification	

The following information is gathered for research purposes only and will not be used to identify you in any way. Please be as honest as possible in your answers and where more than one option is given, **tick** the appropriate box.

1. REFERRAL ROUTE:

Came by self	☐	Sent by family	☐
Transfer from hospital	☐	Transfer from prison	☐
Referred by GP	☐	Referred by a consultant	☐
Ordered by Court	☐		

If other, please specify:……

2. ETHNICITY:

White British ☐ White Caribbean ☐ Indian ☐

!

1

Other Asian	☐	Other Black	☐	Irish	☐
White & African	☐	Pakistani	☐	Caribbean	☐
Chinese	☐	Other White	☐	Other Mixed	☐
Bangladeshi	☐	African	☐	Other	☐

If other, please specify:…….

3. EMPLOYMENT:

Full-time employed	☐	Part-time employed	☐
Unemployed	☐	Other	☐

4. LEVEL OF EDUCATION REACHED:

No formal qualifications	☐	GCSE / O-Level	☐
A-Level	☐	Vocational qualifications (e.g. HND, NVQ)	☐
Undergraduate Degree	☐	Postgraduate Degree	☐
Higher	☐	Other	☐

Do you hold a current driving license? Yes ☐ No ☐

Do you usually drive to the clinic to collect your methadone?

Yes ☐ No ☐

SMOKING BEHAVIOUR

5.1 Do you smoke cigarettes or tobacco?

Yes ☐ No ☐ *Please specify which*…….

(if the answer is no, disregard the remaining questions)

5.2. How many or how much do you smoke per day? Number cigs/day ……

ALCOHOL USE BEHAVIOUR

6.1 Do you DRINK alcohol?

Yes ☐ No ☐ *Please specify which*…….

(if the answer is no, disregard the remaining questions)

6.2. How many units of alcohol do you drink per day (1 unit = 1 small glass of wine; half a pint of beer; 1 can strong lager or Cider = 3 unit)

……

6.3. When was the last time that you drank alcohol?

in last 24 h ☐ ; in last week ☐ *Please specify how much you drank*…….

This questionnaire **The Alcohol Use Disorders Identification Test (AUDIT)** Is about your use of alcohol. Your answers will remain confidential so please be honest. Place an X in one box that best describes your answer to each question.

Questions	0	1	2	3	4	
1. How often do you have a drink containing alcohol?	Never	Monthly or less	2-4 times a month	2-3 times a week	4 or more times a week	
2. How many drinks containing alcohol do you have on a typical day when you are drinking?	1 or 2	3 or 4	5 or 6	7 to 9	10 or more	
3. How often do you have six or more drinks on one occasion?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
4. How often during the last year have you found that you were not able to stop drinking once you had started?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
5. How often during the last year have you failed to do what was normally expected of you because of drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
6. How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
7.How often during the last year have you had a feeling of guilt or remorse after drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
8. How often during the last year have you been unable to remember what happened the night before because of your drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
9. Have you or someone else been injured because of your drinking?	No		Yes, but not in the last year		Yes, during the last year	
10.Has a relative, friend, doctor, or other health care worker been concerned about your drinking or suggested you cut down?	No		Yes, but not in the last year		Yes, during the last year	
					Total	

Methadone Intake

7.1 Are you currently receiving methadone?

Yes ☐ No ☐

7.2 What is the dose of methadone you are receiving?

....

7.3 How long have you receiving methadone maintenance treatment?

More than a month ☐ Less than a month ☐

7.4 Have you ever taken extra methadone on top of your prescription?

Yes ☐ No ☐

If yes what is the usual amount that you take on top .....amount in mg

How often to do consume extra methadone?
Every day ☐ **most days** ☐ **Once a week** ☐ **less frequently** ☐

When was the last time that you missed a normal daily dose of prescribed methadone?

In last week ☐ in last month ☐ never ☐ other (say what happened)……

Other medications

8.1 Are you currently prescribed medications other than your methadone?

Yes ☐ No ☐

8.2 What is the medication prescribed for?

.....

8.3 What is the dose of the medication prescribed?

……

The following questionnaire is concerned with any withdrawal symptoms that you may experience in the last 24 hours

The Short Opiate withdrawal Scale (SOWS)

Please put a check mark in the appropriate box if you have suffered from any of the following conditions in the last 24 hours:

	None (0)	Mild (1)	Moderate (2)	Severe (3)
Feeling Sick				
Stomach Cramps				
Muscle Spasms				
Feelings of Coldness				
Heart Pounding				
Muscular Tension				
Aches and Pains				
Yawning				
Runny Eyes				
Insomnia/Problems Sleeping				

Appendix E

Treatment Outcomes Profile

Client ID | D.O.B. (dd/mm/yyyy) | Name of keyworker

TOP interview date (dd/mm/yyyy) | Gender: M ☐ F ☐ | Treatment stage: Start ☐ Review ☐ Exit ☐ Post-treatment exit ☐ | Total for NDTMS return

Section 1: Substance use (Use NA only if information is not disclosed or not answered)

Record the average amount on a using day and number of days substances used in each of past four weeks

	Average		Week 4	Week 3	Week 2	Week 1	Total
a Alcohol		units/day	0–7	0–7	0–7	0–7	0–28
b Opiates/opioids (illicit)*		g/day	0–7	0–7	0–7	0–7	0–28
c Crack		g/day	0–7	0–7	0–7	0–7	0–28
d Cocaine		g/day	0–7	0–7	0–7	0–7	0–28
e Amphetamines		g/day	0–7	0–7	0–7	0–7	0–28
f Cannabis		spliff/day	0–7	0–7	0–7	0–7	0–28
g Other problem substance? (name.....)		g/day	0–7	0–7	0–7	0–7	0–28

*Includes street heroin and any non-prescribed opioid, such as methadone and buprenorphine

Section 2: Injecting risk behaviour (Use NA only if information is not disclosed or not answered)

Record number of days client injected non-prescribed drugs in past four weeks
(if no, enter zero and 'N', and go to section 3)

	Week 4	Week 3	Week 2	Week 1	Total
a Injected	0–7	0–7	0–7	0–7	0–28
b Inject with needle or syringe used by someone else?	Yes ☐ No ☐				Enter 'Y' if any yes, otherwise 'N'
c Inject using a spoon, water or filter used by someone else?	Yes ☐ No ☐				

Section 3: Crime (Use NA only if information is not disclosed or not answered)

Record days of shoplifting, drug selling and other categories committed in past four weeks

	Week 4	Week 3	Week 2	Week 1	Total
a Shoplifting	0–7	0–7	0–7	0–7	0–28
b Drug selling	0–7	0–7	0–7	0–7	0–28
c Theft from or of a vehicle	Yes ☐ No ☐				Enter 'Y' if any yes, otherwise 'N'
d Other property theft or burglary	Yes ☐ No ☐				
e Fraud, forgery and handling stolen goods	Yes ☐ No ☐				
f Committing assault or violence	Yes ☐ No ☐				Enter'Y'or'N'

Section 4: Health & social functioning (Use NA only if information is not disclosed or not answered)

a Client's rating of psychological health (anxiety, depression, problem emotions and feelings)

Poor 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 Good — 0–20

Record days worked and at college or school for the past four weeks

	Week 4	Week 3	Week 2	Week 1	Total
b Days paid work	0–7	0–7	0–7	0–7	0–28
c Days attended college or school	0–7	0–7	0–7	0–7	0–28

d Client's rating of physical health (extent of physical symptoms and bothered by illness)

Poor 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 Good — 0–20

Record accommodation status for the past four weeks

e Acute housing problem	Yes ☐ No ☐	Enter'Y'or'N'
f At risk of eviction	Yes ☐ No ☐	Enter'Y'or'N'

g Client's rating of overall quality of life (able to enjoy life, gets on with family and partner, etc)

Poor 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 Good — 0–20

TOP v1.4 Dec 2009

Appendix F

Institute of Psychiatry

at The Maudsley

KING'S College LONDON Founded 1829

University of London

Title of Research: Alcohol use patterns in patients receiving methadone treatment

Name of Researcher: Basma Alharthy

Participants Identification No. for the study

Date

The following information is gathered for research purposes only and will not be used to identify you in any way. Please be as honest as possible in your answers and where more than one option is given, **tick** the appropriate box.

1. Demographics

A. Age

B. Gender

Female ☐
Male ☐

C. Referral route

Came by self ☐
Transfer from hospital ☐
Referred by GP ☐
Ordered by Court ☐
Sent by family ☐
Transfer from prison ☐
Referred by a consultant ☐

If other, please specify:.....

D. Ethnic Background

White British ☐
Other Asian ☐
White Caribbean ☐
Other Black ☐
Indian ☐
Irish ☐

White & African	☐	Pakistani	☐	Caribbean	☐		
Chinese	☐	Other White	☐	Other Mixed	☐		
Bangladeshi	☐	African	☐	Other	☐		

E. Employment

Full-time employed	☐	Part-time employed	☐	
Unemployed	☐	Other	☐	

F. Level of Education

No formal qualifications	☐	GCSE / O-Level	☐	
A-Level	☐	Vocational qualifications (e.g. HND, NVQ)	☐	
Undergraduate Degree	☐	Postgraduate Degree	☐	
Higher	☐	Other	☐	

If other, please specify:.....

G. Driving

	Yes	No
Do you hold a current driving license?		
Have you ever held a driving license?		
Were you ever convicted for drink driving?		
Were you ever convicted for drug driving?		
Do you drive to the drug treatment clinic?		

If other, please specify:.....

II. Smoking Behavior

Do you smoke cigarettes or tobacco? *Please specify*	Yes	No

How many or how much do you smoke per day? Number cigs/day.....

Do you smoke cannabis with tobacco?	Yes	No

How many or how much do you smoke per day? Number cigs/day.....

III. Methadone Treatment

A. When were you first prescribed methadone?

.....

B. Have you stopped and needed to be restarted on methadone?

Yes ☐ N☐

Please specify .....

C. What is the dose of methadone you are receiving?

.....

D. Have you ever taken extra methadone on top of your prescription?

Yes ☐ No ☐

If yes what is the usual amount that you take on top .....amount in mg

E. How often to do consume extra methadone?

Every day ☐ most days Once a week ☐ less frequently ☐

F. Do you find that your alcohol intake tend to increase if missed your methadone daily dose?

Yes ☐ No ☐

G. How often do you visit the clinic to collect your methadone?

Daily ☐
Weekly ☐
Once every two weeks ☐
Other ☐
If other, please specify

H. When was the last time that you missed a normal daily dose of prescribed methadone?

In last week ☐ In last month ☐ never ☐ other .....

4

1. Leeds Dependence Questionnaire - LDQ

The following questions are about the importance of alcohol or other drugs in your life.
Think about the main substance you have been using over the **last 4 weeks** and tick the closest answer to how you see yourself

	Never	Sometimes	Often	Nearly Always
	0	1	2	3
1. Do you find yourself thinking about when you will next be able to have another drink or take more drugs?				
2. Is drinking or taking drugs more important than anything else you might do during the day?				
3. Do you feel that your need for drink or drugs is too strong to control?				
4. Do you plan your days around getting and taking drink or drugs?				
5. Do you drink or take drugs in a particular way in order to increase the effect it gives you?				
6. Do you drink or take drugs morning, afternoon and evening?				
7. Do you feel you have to carry on drinking or taking drugs once you have started?				
8. Is getting an effect more important than the particular drink or drug you use?				
9. Do you want to take more drink or drugs when the effects start to wear off?				
10. Do you find it difficult to cope with life without drink or drugs?				

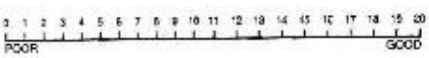
IV. History

A. Family History

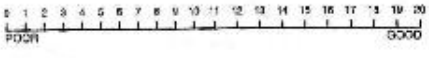
	Yes	No	Please specify
1. Do any of your family members have alcohol problem?			
2. If yes, have they ever received treatment for their alcohol problem?			
3. Do any of your family members have substance dependence problem?			
4. If yes, have they ever received treatment for their substance dependence problem?			

B. Current Physical and Psychological health

1. CLIENT'S RATING: PHYSICAL HEALTH
(Extent of physical symptoms and ailments) (in illness)



2. CLIENT'S RATING: PSYCHOLOGICAL HEALTH
(Anxiety, depression, problem emotions and feelings)



C. Prescribed Medication

1. Are you currently prescribed medications other than your methadone?

Yes ☐ No ☐

2. Prescribed medication list (Please specify)

Drug	Prescribed for	Name	Dose
Benzodiazepine			
Antipsychotic			
Antidepressants			

D. Drug Use

Record the number of using days in each of the past four weeks, and the average amount used on a using day

Substance	Describe your use in the past 24 hours	WEEK 1 (0-7)	WEEK 2 (0-7)	WEEK 3 (0-7)	WEEK 4 (0-7)	Average Per day	Total (0-28)
OPIOIDS (ILLICIT)						G	
CRACK						G	
COCAINE						G	
AMPHETAMINES						G	
CANNABIS						G	
OTHER						G	

V. Current Alcohol use

A. Do you consume Alcohol on a regular basis?

Yes ☐ No ☐

B. How many units of alcohol do you drink per day

(1 unit = 1 small glass of wine; half a pint of beer; 1 can strong lager or Cider = 3 unit)

.....

C. When did you last consume an alcoholic beverage?

.....

Please specify how much you.....

D. Describe your alcohol consumption for the past 72 hours

	Describe your use in the past 72 hours	WEEK 1 (0-7)	WEEK 2 (0-7)	WEEK 3 (0-7)	WEEK 4 (0-7)	Average Per day	Total (0-28)
ALCOHOL	First drink Last drink					Units	

E. Alcohol Use Disorders Identification Test (AUDIT)

The following questions are about your use of alcohol in the past year. Your answers will remain confidential so please be honest. Place an X in one box that best describes your answer to each question

Questions	0	1	2	3	4	
1. How often do you have a drink containing alcohol?	Never	Monthly or less	2-4 times a month	2-3 times a week	4 or more times a week	
2. How many drinks containing alcohol do you have on a typical day when you are drinking?	1 or 2	3 or 4	5 or 6	7 to 9	10 or more	
3. How often do you have six or more drinks on one occasion?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
4. How often during the last year have you found that you were not able to stop drinking once you had started?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
5. How often during the last year have you failed to do what was normally expected of you because of drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
6. How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
7.How often during the last year have you had a feeling of guilt or remorse after drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
8. How often during the last year have you been unable to remember what happened the night before because of your drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
9. Have you or someone else been injured because of your drinking?	No		Yes, but not in the last year		Yes, during the last year	
10.Has a relative, friend, doctor, or other health care worker been concerned about your drinking or suggested you cut down?	No		Yes, but not in the last year		Yes, during the last year	
					Total	

VI. Breathalyzer test

A. Do you get breathalysed when you collect your methadone prescription?

Yes ☐ No ☐

Please specify.....

B. How frequently do you fail the breathalyser test?

All the time ☐ Rarely ☐

Most of the time ☐ Not at all ☐

About half of the time ☐

C. When was the last time you were breathalysed?

.....

D. When was the last time you failed your breathalyser test?

.....

E. What was the breathalyser reading?

.....

F. Describe your alcohol consumption before you were breathalysed *(When was your last alcohol consumption and how much)?*

.....

G. Were you breathalysed more than once?

Yes ☐ No ☐

Please specify .....

If yes, how long did you wait between your Breathalyser tests?

.....

H. Do you find yourself having to change your drinking pattern to avoid failing breathalyser test?

Yes ☐ No ☐ *Please specify*.....

VII. Short Withdrawal Scores: The following questionnaires are concerned with any opiates or alcohol withdrawal symptoms that you may experience in the last 24 hours

A. The Short Opiate withdrawal Scale (SOWS)

	None (0)	Mild (1)	Moderate (2)	Severe (3)
Feeling Sick				
Stomach Cramps				
Muscle Spasms				
Feelings of Coldness				
Heart Pounding				
Muscular Tension				
Aches and Pains				
Yawning				
Runny Eyes				
Insomnia/Problems Sleeping				

B. The Short Alcohol Withdrawal Scale (SAWS)

	None (0)	Mild (1)	Moderate (2)	Severe (3)
Anxious				
Sleep disturbance				
Problems with memory				
Nausea				
Restless				
Tremor (shakes)				
Feeling confused				
Sweating				
Miserable				
Heart pounding				

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